Arsenic and trace metals in hair, nails and blood of villagers from the vicinity of a gold mine in Tanzania

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Preface

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Summary

Tanzania has had mining on a large scale since 1998. The North Mara Gold Mine is located in Tarime District in the north-western part of Tanzania. Recent studies in the North Mara area have indicated that mining activities release trace elements to the surrounding environment, potentially exposing the local population.

The aim of the study was to clarify whether villagers living in the vicinity of the North Mara Gold Mine were exposed to trace elements at levels sufficiently high to be detected and quantified in hair, nails and/or blood. Influence of age, gender and smoking habits were investigated as well as potential relationships between trace element concentrations in hair and nails.

Samples of hair, nail and blood were collected from 63 subjects from the villages Nyangoto, Kewanja, Matongo, Nyarwana, Nyakunguru, Weigita and Nkerege, as well as from a reference group from Dar es Salaam. Concentrations of arsenic (As), antimony (Sb), cadmium (Cd), lead (Pb), zinc (Zn), manganese (Mn), molybdenum (Mo), copper (Cu) and thorium (Th) were quantified in all tissues by inductively coupled plasma mass spectrometry (ICP-MS).

Concentrations of As, Mn and Th in hair and nails were higher in villagers from Tarime District compared to the reference group and normal ranges found in previous studies. There was no suspected exposure to Sb, Cd and Cu as the concentration in both hair and nails were within normal ranges, but the concentration of Zn in hair and nails indicated that some subjects suffer from Zn deficiency. The high and low concentrations of As and Zn respectively, may potentially lead to health impairments associated with As toxicity and Zn deficiency. The trace element concentrations in blood were not higher than normal ranges found in non-exposed populations elsewhere.

Gender and age influenced the concentration of As, Pb, Cu and Th in hair, but not in nails. The concentration of As in men was significantly influenced by age and smoking for hair and nails respectively. Accumulation of trace elements did however appear to be more strongly affected by the village of habituation than any other factor. Correlation between matched hair and nail samples were found for the elements As, Cd, Zn and Th. There is a clear need to clarify possible health impairments associated with the elevated As concentrations observed as well as accurately identify sources of exposure.

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Abbreviations

AAS - Atomic Absorption Spectroscopy

As – arsenic

As^{3+} – arsenate / trivalent arsenic

As^{5+} – arsenite / pentavalent arsenic

BD - Becton, Dickinson and Company

BGC - Barrick Gold Corporation

Cd - cadmium

CN - cyanide

Co – cobalt

Cu – copper

EPA - Environmental Protection Agency

GDP - gross domestic product

GLM - generalized linear model

Hg – mercury

IARC - International Agency for Research on Cancer

ICP-MS - Inductively Coupled Plasma Mass Spectrometry

ICP-OES - Inductively Plasma Optic Emission Specrtometry

INAA - Instrumental Neutron Activation Analysis

Mn – manganese

Mo - molybdenum

NCA – Norwegian Church Aid (Kirkens Nødhjelp)

NGO - non-governmental organization

NMGM – North Mara Gold Mine

NTNU - Norwegian University of Science and Technology (Norges teknisk-naturvitenskapelige universitet)

Pb - lead

PCA - principal component analysis

R-SH - sulphydryl groups

Sb – antimony

Se - selenium

SE – standard error

Th - thorium

UDSM – University of Dar es Salaam

UiO- University of Oslo

UMB – Norwegian University of Life Sciences

US-EPA - U.S. Environmental Protection Agency

WHO - World Health Organization

Zn – zinc

1. Introduction

1.1. Global gold mining and the North Mara Gold Mine

Gold (Au) is considered an important reserve asset by most national banks, even though it no longer forms the basis of international financial systems. The metal has vital functions in many areas of everyday life; e.g. medical applications, pollution control, airbags, mobile phones, laptop computers and space technology (Enriquez and Drummond 2007). The total amount of gold that has ever been mined is estimated at about 164 000 metric tons, and developing countries accounts for roughly two-thirds of the global gold production (Butt and Hough 2009). Gold production can result in the development of electricity, water supply and infrastructure in mined areas, generates export revenue, employment, and it provides tax income for governments (Enriquez and Drummond 2007). While mining contributes to around 1% of global gross domestic product (GDP), it consumes 7-10% of global energy and is responsible for 13% of sulphur dioxide emissions (Bebbington et al. 2008). The global mining industry has been moving towards more sustainable processes in recent years due to public concern over long-term environmental impacts (Mudd 2007). Environmental, economic and social developments are important factors for sustainable management of mining operations (Amankwah and Anim-Sackey 2003).

The demand of gold has been increasing in developed countries, and the mineral is traded over great distances, particularly in Africa (Butt and Hough 2009). Gold has overtaken agricultural products as Tanzania's largest export and accounts for around 44% of the exports today. Tanzania has had mining on a large scale since 1998, and the government has the vision that the mineral sector will contribute to 10% of the GDP by 2025 (Imparato 2010). North Mara Gold Mine is located in one of the active mining areas of the country in Tarime District, approximately 100 km east of Lake Victoria and 20 km south of the Kenyan border (Anonymous 2009).

Production of gold and other metals may impact the local communities and the environment (Amankwah and Anim-Sackey 2003). Mining generally involves processing large amounts of mineral-rich rock to extract the element or elements of interest. Mine dumps and tailings is the material left over from the ore processing, and there are a number of fundamental issues and concerns with ensuring the sustainability of mining (Dolgopolova et al. 2006). Gold-rich ore typically contains high concentrations of other trace elements, and the elements present in the ores are released during the smelting process and from deposits (Roy and Saha 2002). The natural presence of ore elements is the reason for their release to the environment, not any addition of

e.g. As in the extraction process. Ground rock or dust from mine tailings is very susceptible to chemical weathering, and minerals contained in the dust easily oxidise and dissolve when exposed to oxygen and water (Dolgopolova et al. 2006).

Increasing production of waste rock is due to the trend towards open large-scale mining, and more complex ores are being developed (Mudd 2007). There are obvious challenges to retain waste associated with open-pit mining due to the large volumes of rock and process water involved. One common solution is to store the extracted minerals under large water-covered artificial lakes. The suitability of this solution clearly depends on the ability to control seepage and other releases to the surrounding environment. Previous studies have shown that the toxicity and mobility of metals depend strongly on their specific forms or binding state (Kashem et al. 2007). Seepage would typically have low pH and contain very high concentrations of elements not extracted by the process or immobilised in the artificial lake. Aquatic ecosystems and local populations may be impacted due to discharges from mining activity (He et al. 1998; Guo et al. 2011). Mining have been identified as the main cause of soil contamination of copper (Cu), lead (Pb), cadmium (Cd) and zinc (Zn) in Zambia (Tembo et al. 2006).

The North Mara Gold Mine consists of open pit deposits, and the process used for gold extraction is gold cyanidation (Anonymous 2009). Recent studies in the North Mara area have indicated that the mining activity causes the release of trace elements to the surrounding environment, some of which have been detected in elevated concentrations in water (Table 1), soil and sediments (Almås et al. 2009). Two sites were excluded from Table 1 because inflow from contaminated water from the mine was unlikely due to the topography of the land.

Table 1. Trace element concentrations in water (µg/L) from areas surrounding North Mara Gold Mine (Almås et al. 2009). Minimum and maximum values are shown for all sampled locations excluding spill-sites and control, followed by min-and max values for spill-sites only. Control site value and the WHO guidelines for safe drinking water concentrations (µg/L) are shown for comparison.

	No. of samples	As	Cd	Cu	Pb	Th	Zn
Water	8	0.9-1142	<LD-0.26	0.8 - 5.3	<LD - 0.14	<LD - 0.2	1.0 - 17.5
Spill site	3	307 - 8449	108 - 224	1670-4467	0.6 - 7.8	34-169	43 473 - 94 608
Control site	1	0. 7	<LD	0.5	0.1	0.1	3.6
WHO guideline		10	3	2000	10	-	3000*

- No required WHO guideline value.
* No required guideline value, but a Zn level <3000 µg/L in water has been proposed (WHO 2008).
<LD: below level of detection

In general, there were not very high contents of trace elements in soil. Almås et al. (2009) did not find elevated levels of mercury (Hg), otherwise commonly found associated with gold mining. An accidental spill occurred from the mine in May 2009 when water seeped from a mine rock storage facility into the Tigithe River. Water samples from the sites where the spill took place indicated that trace elements were related to the mining activity at North Mara Gold Mine. Concentrations of As, Cu, Cd, Pb and Zn in water and/or spill sites exceeded the WHO recommended drinking water guidelines (Table 1, WHO 2008). There is no generally accepted standard for thorium (Th), but the element appeared to be present at high concentrations in the seepage from the mine. Apart from the accidental spill site, only As was found at concentrations exceeding WHO guideline values in environmental samples (Table 1).

There were no differences in trace element concentration between top-soil and sub-soil at most sites in the study of Almås et al. (2009), which indicate that the presence of the relevant trace elements in soil in Tarime District is probably not due to atmospheric deposition. The situation in North Mara may cause unwanted exposure for wildlife, livestock, vegetation and humans to trace elements such as arsenic. There is clearly a need to clarify whether observed increased environmental concentrations reflect a general situation in Tarime District and has led to exposure of As for villagers living in the area.

The first biomonitoring study of metal exposure in the African Copperbelt reported elevated concentrations of trace elements in subjects living in the vicinity of mines in Congo (Banza et al. 2009). The elements As, Cd, Cu and Pb were found to be significantly higher in urine samples of exposed population compared to the control group. Several other studies have found high concentrations of As in drinking waters as well as human samples from areas near mines, e.g. in the inhabitants living in the vicinity of mining operations in Thailand, Slovakia and Southwest China (Roy and Saha 2002; Rapant et al. 2006; Liu et al. 2011a). Another study from a mining area found no correlation between As concentration in drinking waters and human samples in Ghana (Asante et al. 2007). The As concentration were low in water supplies and high in urine samples, and a suggested source was vegetables grown on contaminated soil in the vicinity of the mining area (Asante et al., 2007).

1.2. Trace elements and human exposure

Humans are exposed to trace elements through naturally occurring and anthropogenic releases. The routes of exposure include inhalation of contaminated dust, ingestion of drinking water and dust, and consumption of plants and animals (Orloff et al. 2009). Dust from mining areas

frequently contain high concentration of metals and may cause exposure when carried away from the mining areas (Dolgopolova et al. 2006). Vegetables occasionally contain high levels of trace elements, and numerous reports have documented that plants grown on metal contaminated soil can accumulate elevated metal concentrations (Wei et al. 2011). Human exposure to metals may increase through ingestion of vegetables with high concentrations of trace elements in areas with long history of mining.

Humans are exposed to As through air, drinking water and food, and important food sources are fish and seafood (Silvera and Rohan 2007). Concentrations of organic As in seafood appear to have no negative health effects as the relevant forms are not readily accumulated by humans (Cleland et al. 2009). Arsenic-rich coal is often used for indoor stoves (Vahter 2009), and it is commonly used for heating and cooking in Africa (Shraim et al. 2003). Arsenic has been used for medicinal applications and as a suicidal or homicidal agent (Chouhan and Flora 2010). Various organic As compounds and herbal products are still used in human medicine in Africa, and organic As is used to treat African sleeping-sickness (Roy and Saha 2002). The element is non-essential, but the level of As bioavailability is not a threat for human health under normal ecological conditions (Roy and Saha 2002). Absorption of inorganic As mainly occurs through inhalation in occupational settings or ingestion in the general population (Buchet et al. 1999). Exposure to As has been associated with a range of adverse effects, including injure to lungs, liver, brain and reproductive system (Kapaj et al. 2006). Arsenic has been categorized as a class I carcinogen since 1980 by The International Agency for Research on Cancer (IARC) (Silvera and Rohan 2007). Comparison of different epidemiologic studies of trace element exposure and cancer risk has signified a positive correlation between As exposure and cancer in skin, lungs and bladder (Smith et al. 2000; Russi et al. 2005).

Other elements or metals commonly associated with mining activities and possible exposure for local inhabitants are antimony (Sb), manganese (Mn), Pb, Zn, Cu and Th (Ting et al. 1996; Nowak and Chmielnicka 2000; Samanta et al. 2004; Matthies et al. 2011).

Sb is commonly released as a result of mining processes (Rapant et al. 2006; Liu et al. 2011a). The non-essential element is a metalloid similar to As both physically and chemically (Wei et al. 2011), and Sb has been classified as a priority pollutant by the US Environmental Protection Agency (US EPA) (Liu et al. 2011a). Elevated concentrations of Sb have been detected in human hair following exposure from mining activity (Laura Barbieri et al. 2011) and as a consequence of smoking habits (Serdar et al. 2009). Quantitative data on human absorption through inhalation or ingestion of Sb is not available (Tylenda and Fowler 2007).

Cd concentrations were low in environmental samples from North Mara (Table 1), although present at the spill sites. Sources of Cd generally include inhalation of aerosols and ingestion of food with high dietary fibre content (Nordberg et al. 2007b). The metal is non-essential, and its ability to bind to metallothionein (MT) is the reason for the long half-life of 10-30 years. Human exposure to Cd may cause heart disease, diabetes and renal dysfunction (Massadeh et al. 2011), and the metal has been classified as a human carcinogen by the IARC (Russi et al. 2005). Mines have been associated with Cd contamination, and high Cd levels were detected in human urine following mine leakage in Japan. Adverse effects such as osteomalacia (softening of the bones), arose in the exposed population (Nordberg 2009).

Inorganic Pb is the most studied toxic metal, and blood is the most common biological matrix for Pb determination (Skerfving and Bergdahl 2007). Exposure to the non-essential metal has been a problem for humans worldwide, and paints in houses and leaded gasoline have been important anthropogenic sources (Nordberg et al. 2007a). Environmental regulations has significantly reduced or eliminated the use of lead in non-battery products, including gasoline, paints, and water systems. In areas where the air Pb level is low, food is the dominating source of Pb uptake (Skerfving and Bergdahl 2007). Leaded gasoline has been phased out successfully in most nations, including sub-Saharan countries (Skerfving and Bergdahl 2007).

There was very high concentration of Zn in the spill from North Mara Gold Mines (Table 1). Exposure through inhalation is usually highest in urban and industrial areas, but human health effects following such exposures are rare (Sandstead and Au 2007). The mineral is essential and has various functions; including an important role in protein synthesis, gene-regulation, and for the immune function. Zn can enter stream water from active or inactive mines, and it is normally found in association with other metals such as Cu and Pb in ores (Guo et al. 2011). Zn deficiency affects people in the developing world, and a conservative estimate has suggested 20.5% of the world's population to be at risk for inadequate Zn intake (Wuehler et al. 2005). Zinc deficiency has been found to be associated with cancer risk (Campos et al. 2008).

Mn is known to be related to mining activity and can potentially contaminate the surrounding water (Asante et al. 2007). Food is the major source of Mn intake for humans, and wheat, rice and legumes contain high concentrations of Mn (Bertrandt et al. 2001) The element is essential, but no large-scale deficiency has been reported (Saric and Lucchini 2007). Long-term exposure may result in adverse effects on the central nervous system (CNS) as the primary target and on the lungs as the second target (Wright et al. 2006; Saric and Lucchini 2007).

Cu was present in high concentrations in the spill from North Mara Gold Mines (Table 1). The element has earlier been detected in surrounding soil and in human tissue from areas with mining activity (Georgopoulos et al. 2001; Kleiv and Thornhill 2004; Banza et al. 2009). Cu is an essential element and is required component of more than 70 enzymes (Bertrandt et al. 2001). Food items containing Cu include fish, fruits, cereals, vegetables and organ meats such as liver and kidney. Higher levels of Cu may be found in urban or polluted areas (Georgopoulos et al. 2001). Gastrointestinal disturbances can occur following ingestion of Cu, and the element may cause hemolysis and damage to liver and kidney at very high exposure levels (Ellingsen et al. 2007).

Th was detected in the spill from North Mara Gold Mines (Table 1), and human exposure has earlier been shown to occur at locations close to mining activity (Ting et al. 1996). Th is a non-essential radionuclide, and dust and volcanic eruptions are natural sources of exposure through inhalation. Food and water are sources for human Th exposure through ingestion, and Th isotopes can be detected at low concentrations in human tissue (Rogers et al. 1991). Exposure to Th has been linked to increased incidence of cancer, respiratory diseases and liver damage (Najem and Voyce 1990).

1.3. Indicator tissues

Determination of a trace element profile in human tissue can be used as a biomonitoring tool to investigate the exposure history or assess any deficiency for a particular element in a study population. Concentration in human tissues can indicate the actual exposure and lead to a better assessment of potential health risks.

Hair and nails are metabolically inactive body tissues, and they are useful indicators for some trace elements to which a subject has been exposed to over a period of several weeks to months (Yoshinaga et al. 1990). Elements such as As can be incorporated into the hair and nail due to the rich blood supply to the hair root and nail bed respectively (Orloff et al. 2009). While incorporation into both matrices is almost immediate, nails will by necessity be sampled following a period of growth, sometimes exceeding one year. The concentration of trace elements can be up to 10 times higher in strands of hair compared to corresponding blood samples (Gellein et al. 2008). Low concentrations, small sample volumes and problems in differentiating between endogenous and exogenous deposition are some of the difficulties associated with analyses of hair and nails (Chojnacka et al. 2006). Hair grows approximately 1 cm a month and trace elements incorporates in the hair strands during the growth process (Gellein et al. 2008). Previous studies

have found that hair is a representative tissue for biological monitoring of exposure to As, Sb, Cd, Pb and Cu (Kosanovic and Jokanovic 2011). The Environmental Protection Agency reported that human hair is one of the tissues of choice used for biological monitoring of the highest priority toxic metals and for determining toxic metal exposure (Massadeh et al. 2011).

Human nails consist of keratin-rich proteins, which make it stable and robust (He 2011). Trace elements will be incorporated in nails by binding to sulphydryl (SH) groups. Average growth rate for fingernails has been estimated to be 3-4 mm per month, but the growth differs due to factors such as age, sex and health status (He 2011). The same review concluded that approximately 1 mm of nail sample corresponded to one month of nutritional status. Nails are useful for exposure assessment for trace elements, and they have been used for biological monitoring with increasing frequency (Rodushkin and Axelsson 2000).

Blood and urine reflects the trace element concentration in blood plasma at the time the sample was collected (Gellein et al. 2008). The urine concentration of As will be influenced by diet and contains mainly organic As, making it problematic to evaluate the total body burden of inorganic As (Orloff et al. 2009). Blood is useful as a marker of internal dose and recent exposure to trace elements (Cornelis et al. 1994).

There are a number of chemical analyses and biological approaches to understand the influence of gender and age on contaminant levels and effects in humans, but no systemic study has been conducted to assess the influence of such factors on trace element concentrations (Orloff et al. 2009). Biomonitoring results play an important role in decision-making regarding health impact, and consideration of factors influencing the validity of such data is essential. Gender and age have been found to be discriminatory factors on trace element concentration in hair and nails in several studies (Vance et al. 1988; Nowak and Chmielnicka 2000; Chojnacka et al. 2006). The factors have also been found to influence the blood and urine levels of trace elements, e.g. As, Cd and Pb (Christensen 1995). One study indicated that some essential elements, e.g. Mn, Cu and Zn, tended to be higher in female hair, whereas the toxic metal Pb were more strongly associated with men's hair (Zakrgynska-Fontaine et al. 1998). Toxicokinetics, toxicodynamics and other modulating factors will necessarily depend on gender, thus influencing the accumulation and effects of trace elements in humans (Gochfeld 2007). Women may e.g. excrete trace elements by transferring them to the developing foetus or through excretion into breast milk (Burger 2007). Tobacco smoking has been found to be a contributing factor to higher bioaccumulation in human tissues of some elements such as As, Cd, and Pb (Chiba and Masironi 1992; Mehra and Juneja 2005).

1.4. Objectives

The main objective of the study was to clarify whether villagers from the Tarime District in the vicinity of North Mara Gold Mine, were exposed to trace elements at levels sufficiently high to be detected and quantified in hair, nails and/or blood. There was a special focus on As since elevated levels has been detected in water samples from Tarime District (Almås et al. 2009), and because As exposure is known to cause health impairment in other areas of the world.

The main objective can be broken down into the following sub-goals:

- Were concentrations of As, Sb, Cd, Pb, Zn, Mn, Cu and Th higher in nails, hair and/or blood from villagers in Tarime District compared to the reference group from Dar es Salaam?
- Was the concentration of any of the trace elements sufficiently high or low in villagers to indicate possible health impacts associated with toxicity or deficiency?
- Was there a relationship between the concentrations of each element in hair and nails?
- Did gender, smoking habits and/or age affect trace element concentrations in hair or nails from villagers?
- Was the relative trace element composition different in villagers from Tarime District compared to the reference group from Dar es Salaam?
- What was the best matrix for determination of trace element exposure?

2. Arsenic

Arsenic is a metalloid existing in the earth crust, and slow release of As from rocks and sediments contribute to the flux of As in the environment (Roy and Saha 2002). Sources of As can be natural such as erosion and leaching from geological formations, or anthropogenic sources such as industry and mining activities (Kapaj et al. 2006). Industries using inorganic As and its compounds include wood preservation, glass production, pesticides and electronic manufacturing. Pesticides and insecticides containing As can possibly contaminate the agricultural crop (Chouhan and Flora 2010).

Arsenic is present both in allotropic forms and several ionic forms, and mechanisms of toxicity depends on factors such as nutritional- and health status (Chouhan and Flora 2010). Pentavalent arsenic (As^{5+}) is the least toxic of the inorganic forms, whereas trivalent arsenic (As^{3+}) is more toxic due to its high activity and its ability to amplify genes in mammalian cells (Chouhan and Flora 2010). As^{3+} can bind to sulphydryl groups and thus react with a variety of proteins and inhibit their activity (Roy and Saha 2002).

The most important exposure to As is through drinking water and diet (Figure 1). The metal will be absorbed in the gastro intestinal tract where both trivalent and pentavalent As will be further metabolized (Phan et al. 2010). Meat, dairy products and agricultural food such as cereals, vegetables and rice may contribute to exposure through ingestion. Oral ingestion of organic As is less toxic than inorganic As species since the compounds are less absorbed, detoxified by liver methylation, and easily eliminated through urine (Phan et al. 2010).

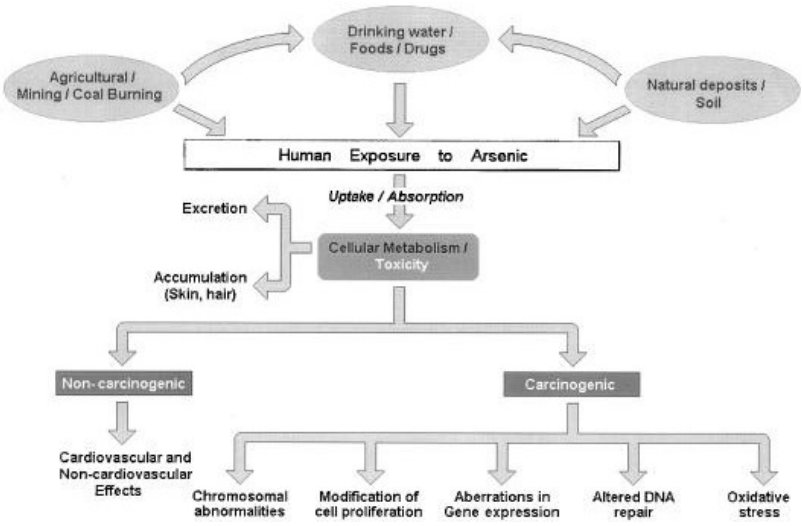


Figure 1. Human exposure and mechanisms of As toxicity; from Roy and Saha (2002).

Arsenical skin lesions are the main sign of arsenicosis (Mao et al. 2010). Exposure to As can cause cardiovascular disease, respiratory system disease and affect memory and intellectual function (Kapaj et al. 2006). Complications for maternal health and child development may occur as inorganic As can pass the placenta. Observed teratogenic effects are iron deficiency in late pregnancy, and interaction with steroid hormones; e.g. oestrogen (Vahter 2009). A study found that subjects with high As body burden or arsenical skin lesions had elevated levels of arsenic in their breast milk samples (Samanta et al. 2007).

The significance of coal burning was evaluated in a study from China, and the findings suggested this unique type of exposure as a major cause of the arsenicosis observed in the area (Shraim et al. 2003). Contaminated drinking water is the major source of environmental As exposure globally. It has been suggested that geogenic sources and its release in groundwater through natural processes was the predominantly cause of As occurrence in groundwater of Bangladesh (Nickson et al. 1998). Some areas were extremely enriched of As with more than 80% of the tube wells with As contamination (Ahmed et al. 2004).

Inorganic As have been found in tube well water in Vietnam, where As concentration exceeded the WHO drinking water guidelines in about 40% of the groundwater samples (Agusa et al. 2006; Nguyen et al. 2009). The groundwater was likely to be the main source as inorganic As was detected in human hair samples (Agusa et al. 2006). Residents of Cambodia have shown signs of arsenicosis due to installations of tube wells in areas known to be of high risk of As enrichment (Buschmann et al. 2007; Sampson et al. 2008).

3. Materials and methods

3.1. Study area

The sampling was conducted in North Mara Region in north-western Tanzania. Villagers were from seven different locations in Tarime district in the vicinity of North Mara Gold Mine (41°S, 20°E) (Figure 2). Tarime is located about 15 km south of the border to Kenya, 15 km northwest of Serengeti National Park and 60 km east of Lake Victoria (Bitala et al. 2009). The seven sub-villages were Matongo, Nyangoto, Nkerege, Weigita, Kewanja, Nyarwana and Nyakunguru (Figure 2). The reference group was subjects living in Dar es Salaam; employees at the Norwegian Church Aid's office.

Figure 2. Map of Tarime District and North Mara Gold Mine. The villages Nkerege, Weigita, Nyakunguru, Matongo, Nyarwana, Nyangoto and Kewanja are marked in red. Open pits as part of the mining activity can be seen North and South of Kewanja. There are other rivers in the area (see Figure 3); modified from maps and data from Foundation HELP and Google Maps. Map of Tanzania from www.travel.state.gov.

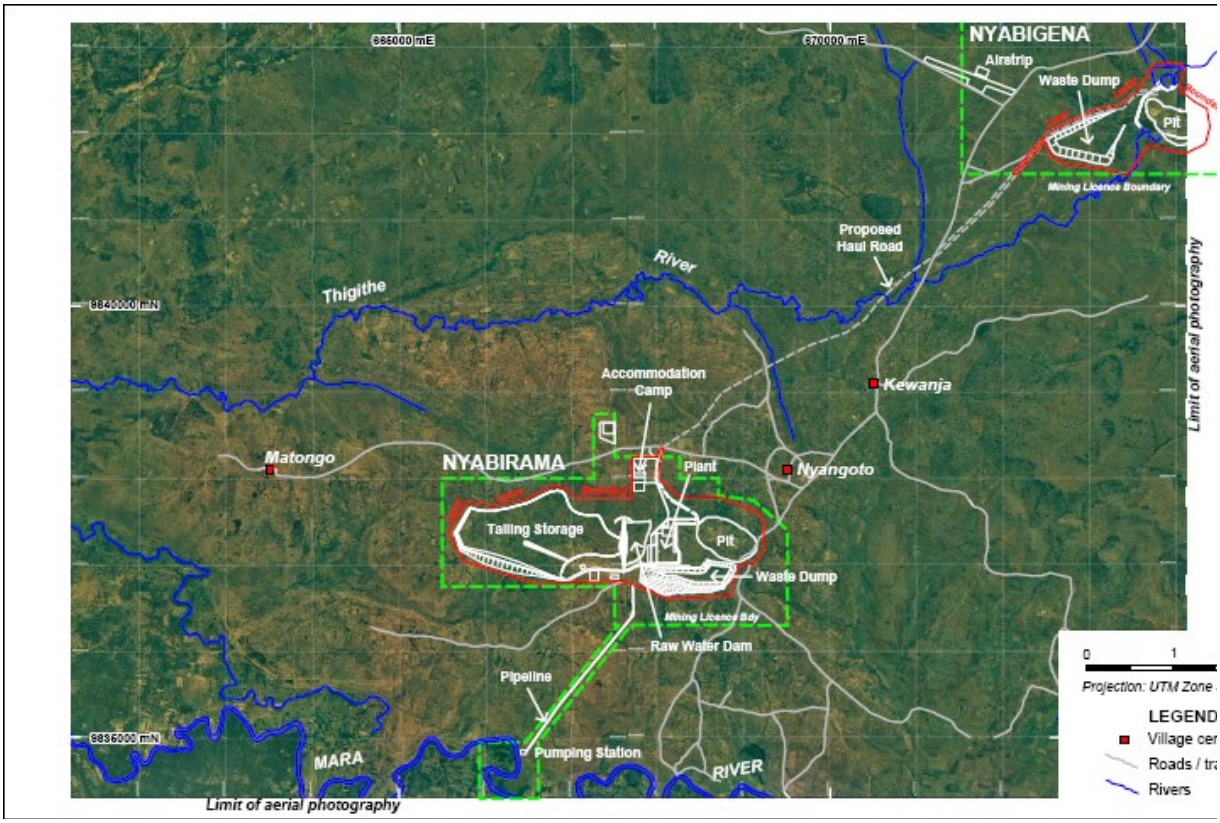


Figure 3. Terrain map of North Mara Gold Mine area; villages from current study: Matongo, Nyangoto and Kewanja; - - - = Nyabirama- and the Gokona-Nyabigena pit, North Mara Gold Mine.

3.2. Equipment

A stainless scissors was disinfected prior collection of hair and nail samples. All equipment for blood sampling was purchased at Puls in Oslo (www.puls-norge.no). The vacuum tubes for 6 mL blood samples for trace element analysis were Vacutainer Plus EDTA 7 mL, 13 x100 mm (Becton, Dickinson and Company (BD), NJ, USA, Figure 4a). The tubes have minimal content of trace elements in order to avoid contamination. EDTA is an anticoagulant, and the blood could therefore be stored for a long time with no risk of coagulation. A cannula was used for obtaining blood samples (Eclipse cannula 21Gx1 1/4", 0.8 mm x 32 mm; BD, NJ, USA, Figure 4b). The needle had a safety shield in order to avoid needle injuries, a known risk for healthcare workers during blood sampling (Lymer et al. 1997).

a

b

Figure 4. (a) BD Vacutainer Plus EDTA and (b) BD Eclipse cannula. From www.puls-norge.no

Blood samples may have been taken from participants who are HIV positive as Northwest Tanzania has an overall seroprevalence of 7.3% HIV-1 infected people (Shao et al. 1994). Co-infection of HIV and the virus hepatitis B or C has also been observed (Nagu 2008). Consequently, appropriate precautions were taken during collection, handling, storage and transportation of blood samples. Regional Committees for Medical Research Ethics (REK) was contacted for approval to conduct the study (Appendix 3).

3.2. Selection of subjects and sampling

Samples were collected in January 2009 in Bunda located Southwest in Mara Region, Tanzania. Local bishops informed the villagers about the importance of the study, and volunteers for sample collection were transported with three buses from the villages in Tarime District. Hair, nail and blood samples were taken from subjects from each of the seven villages around the North Mara Gold Mine. The selection of persons ranged from children to old people of both genders (Table 2). The subjects got information prior the sampling about the objective of the study. Rooms were rented at a local hotel to cater for villagers and for the sampling procedure. Personal information was obtained through a questionnaire in Swahili; including place of residence, gender, age, smoking habits, occupation (Appendix 4). Analphabetic subjects got assistance with filling in the forms.

The equipment for blood sampling was set up and prepared in a clean room and the subjects given individual test numbers. A local doctor assisted with the blood test sampling. A needle holder compatible with all BD needles was reused for the venous blood sampling (Vacutainer one use holder; BD, NJ, USA). Reusable tourniquets (adult 25 mm and infant 10 mm; BD, NJ, USA) with a compressing device were applied above the puncture site for the vessels to become temporarily occluded. The skin was cleaned with disinfectant swabs before inserting the needle, and tight clothes that could constrict the upper arm were removed. Rubber gloves (Emitouch vinyl glove; BD, NJ, USA) were worn during blood collection while the arm rested on a pillow. The tourniquet got released immediately after the insertion, and the sampled tubes were placed in a container for biological samples. Micropore tape attached clean cotton balls to the skin following sampling. The cannulae were released from adapters and discarded directly after single use into a needle disposal box (Sharps Container 1.5 L; BD, NJ, USA).

Single strands of hair were cut as near as possible to the scalp, and fingernail samples were collected using the disinfected scissors. Hair and nail samples were placed into small plastic bags with a closure mechanism and labelled with the test number of the subject.

Collection of hair, nail and blood samples from the reference group was done at the office of Norwegian Church Aid in Dar es Salaam. The equipment and sampling procedure was the same as described previously. The subjects were all from Tanzania, some of whom had been living in Dar es Salaam their entire life. No samples from children were collected from the reference group as the employees were all aged >24 years (Table 2).

Table 2. Number of samples, number of males/females, number of smokers and age distribution (minimum-maximum) for the participants from all locations. * There were no information on gender, smoking or age for three of the participants from Nkerege.

Location	No of samples	Gender (M/F)	No of smokers	Age distribution (min-max)
Dar es Salaam	10	6 / 4	0	24 – 49
Matongo	10	6 / 4	2	7 – 83
Nyangoto	5	5 / 0	2	20 – 69
Nkerege*	12	8 / 1	0	8 – 73
Weigita	13	5 / 8	2	1 – 74
Kewanja	11	8 / 2	3	8 – 72
Nyarwana	6	5 / 1	0	32 – 80
Nyakunguru	5	2 / 3	0	5 – 74
Total	72	45 / 23	9	1 – 83

Personal information has been kept separate and has not been linked to the data presented in this thesis. The linked information was coded to preserve personal identifying information and the anonymity of subjects.

3.3. Storage and transport

Hair and nail samples were stored in the small plastic bags with closure mechanism and the vacuum tubes with blood placed in a leak proof plastic container. All samples were stored at room temperature before and during transportation in a waterproof bag with isolation (H-BIN Biostransport, BIO 02S, 330x260x190 mm; BD, NJ, USA) enclosing a secondary container with absorption mat (H-BIN Biostransport, 300x245x155 mm; BD, NJ, USA). The mat could absorb in case of liquid spills.

All blood samples were kept in a locked refrigerator at 4°C in the lab at the Department of Microbiology at the University of Dar es Salaam. Both secondary container and the waterproof bag was in compliance with EU legislation, and they were used for the transport of samples to Oslo, Norway.

The blood tubes were kept in a locked refrigerator (4°C) at the Norwegian University of Life Sciences (Universitetet for miljø- og biovitenskap, UMB) until analyses. The samples of the reference group from Dar es Salaam were stored and transported the same way as described previously by an employee from NCA's head office in Oslo.

3.4. Trace element analysis

3.4.1. Sample preparation

A washing procedure of the hair and nails had to be completed to remove dust and other external adsorbents. The procedure was comparable with previous studies on trace element analyses in hair and nails (Samanta et al. 2004; Gellein et al. 2008). Single strands of human hair were weighed prior and after the washing procedure. Each sample soaked 30 min at room temperature in the washing solution Triton X-100 (1%, Riedel-de Häen; Seelze, Germany). The Triton X-100 was rinsed off using deionised water (Milli-Q), and samples dried at 50°C for two nights.

An ultrasound bath (Haver USC 200T, Conyers, GA, USA) was used for 10 min to remove contamination tightly adhering to or embedded into the surface of nails. The samples were weighed prior and after washing and thereafter soaked for 60 min at room temperature in 10 mL EDTA (1%, R.P.Normapur AR; Prolabo, France). Deionised water was used twice to rinse off the EDTA. The samples were once more soaked for 30 min in 5 mL EDTA and washed three times with deionised water through filters (S&S Faltenfilter, Ø 125mm, Dassel, Germany). Samples dried at 50°C for two nights.

Most of the hair samples were already very short, so it was no need for cutting into shorter segments. Samples heavier than 0.05 g were cut to achieve a final weight of 0.05 g hair/nails. Plastic gloves and a disinfected scissors were used for the cutting procedure. 0.6 mL of ultrapure nitric acid (HNO_3) was added to the samples as well as 0.5 mL ultrapure water (Milli-Q). The blood samples were kept in a fridge (4ºC) in anticoagulant test tubes pending analysis, and 0.5 mL full blood was added 1.5 mL ultrapure HNO_3.

3.4.2. Digestion and ICP-MS

The metal extraction was done with a high performance microwave reactor by stepwise heating dry material with ultrapure HNO_3 up to 250°C, using an Ultraclave (2 hours, Milestone; Shelton, CT, USA). Preparation of five blanks was done by adding ultrapure HNO_3 only. The samples were transferred to 15 mL Falcon-tubes and diluted with ultra pure water to reach a final volume of 12 mL. Digestion and analysis of blood samples were done the same way as described previously.

The metal concentrations in final extracts were determined at UMB using a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS; Perkin Elmer Sciex Elan, Waltham, MA, USA). Nine trace elements were quantified in all samples, i.e. arsenic (As), antimony (Sb), cadmium (Cd), molybdenum (Mo), lead (Pb), zinc (Zn), manganese (Mn), copper (Cu), and thorium (Th). Five hair and blood samples and seven nail samples were analyzed at the Norwegian University of Science and Technology (NTNU), using HR-ICP-MS (Perkin Elmer Sciex Elan, Waltham, MA, USA). The same elements were analyzed as at UMB except Mn, Cu and Th, for which the number of observations were decreased for some villages. Detection limits were estimated to clarify whether reported values were close to LD (Table 3).

Table 3. Limit of detection (LD), limit of quantification (LQ) and mean values of the blank for all trace elements analyzed ICP-MS.

Element	LD (µg/L)	LQ (µg/L)	Mean blank (µg/L)
As	0.01	0.025	-0.005
Sb	0.004	0.012	0.004
Cd	0.002	0.005	0.001
Mo	0.08	0.26	0.02
Pb	0.04	0.13	0.01
Zn	2.0	6.67	0.49
Mn	0.04	0.13	0.001
Cu	0.79	2.63	0.10
Th	0.004	0.012	-0.002

Some blanks appeared to have negative value because they had slightly lower values than the zero value resulting from the standard curve. Additionally, an estimate was made for the 'true' detection limit in blood samples to clarify whether reported values were at or close to detection limit (Table 4).

Table 4. Examples of 'true' limit of detection in blood samples (µg/L); the 10-percentile of sample weight was used for blood to simulate worst case.

	As	Sb	Cd	Mo	Pb	Zn	Mn	Cu	Th
LD blood (µg/L)	0. 24	0. 096	0. 048	1. 92	0. 96	48. 0	0. 96	19. 2	0. 096

3.7. Statistical analysis

Analyses were performed using JMP 7.0 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). Data for each element and matrix were tested for homogenous variances between groups using Levene's test, and data were log-transformed whenever required (Keyes and Levy 1997). The concentration of all elements in each matrix was evaluated using one-way ANOVA with location as factor (Dalgaard 2008). Whenever the ANOVA was significant, Dunnett's post-hoc test was used to determine differences between values in subjects from the reference group and the seven villages. If variances were not homogenous even following transformation, a non-parametric Kruskal-Wallis test was used (Ruxton and Beauchamp 2008). Values from all villages were thereafter compared with the reference group using Bonferroni-corrected Wilcoxon test with a significance level of 0.007 (0.05/7) for rejection of H_0. The significance level was in all other cases set at 0.05 for rejection of H_0: no difference between groups.

A general linear model (GLM) was performed to clarify whether age, gender and/or smoking affected trace element accumulation for each element and matrix (Liu et al. 2011b).

All elements had significant different variances according to the Levene's test. All trace element concentrations were therefore log-transformed, and values were removed if the trace element concentration was physiological impossible. One half of the value of the respective limit of detection (LD) was substituted for those values below LD and used in statistical analysis; this was never done for more than 20% of samples for any group and always followed by a Kruskal-Wallis analysis (not ANOVA). No statistical analyses were done on Cd and Th in blood and Mo in any biological tissue since >20% of the concentration values were below LD (Helsel 2006).

The nail samples from Nyangoto was analyzed at NTNU, hence no data was available for the elements Mn, Cu and Th as they were not analysed for at NTNU. Consequently, the hair values of such elements from Nyangoto were also excluded from the statistical tests. Single data points were removed from the analyses whenever a sample had concentrations <LD as well as low tissue in-weight to avoid misinterpretation due to multiplication errors.

4. Results

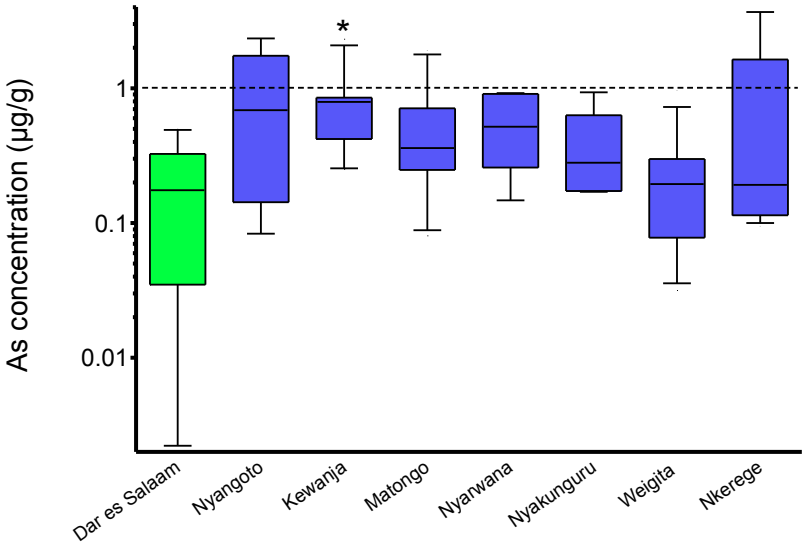
4.1. Arsenic (As)

The variation in As in hair and nails between subjects was higher for some villages than others, and most appeared to have higher concentrations than the reference group from Dar es Salaam (Figure 5).

The variation in As in hair between subjects was higher for Dar es Salaam, Nyangoto and Nkerge than the other locations in Tarime District (Figure 5a). There were significant differences in As-concentrations in hair between the locations (Kruskal-Wallis, DF=7, p=0.02). Levels of As in hair were significantly higher in samples from Kewanja than in the reference group from Dar es Salaam (Bonferroni-corrected Wilcoxon, p=0.0009). The median hair values in villagers from Nkerege and Weigita were comparable to the median of the reference group. The remaining median values of subjects from different locations in Tarime District ranged from 1.5 (Nyakunguru) to 4.4 (Kewanja) times higher than the median of Dar es Salaam.

The variation in As in nails between subjects was high for Nyangoto and low for Nyarwana and Weigita (Figure 5b). There were significant differences in concentrations of As in nails between the locations (ANOVA, DF=7, F=11.04, $p<0.0001$). All villages had significantly higher As-concentrations compared to the reference group from Dar es Salaam (Dunnett; Nyangoto: $p<0.0001$, Kewanja: $p<0.0001$, Matongo: p=0.002, Nyarwana: p=0.03, Nyakunguru: p=0.03, Weigita: p=0.02, Nkerege: p=0.0005). The median nail value in villagers from the different locations in Tarime District ranged from 3.7 (Matongo) to almost 75 (Nyangoto) times higher than the median of Dar es Salaam.

a



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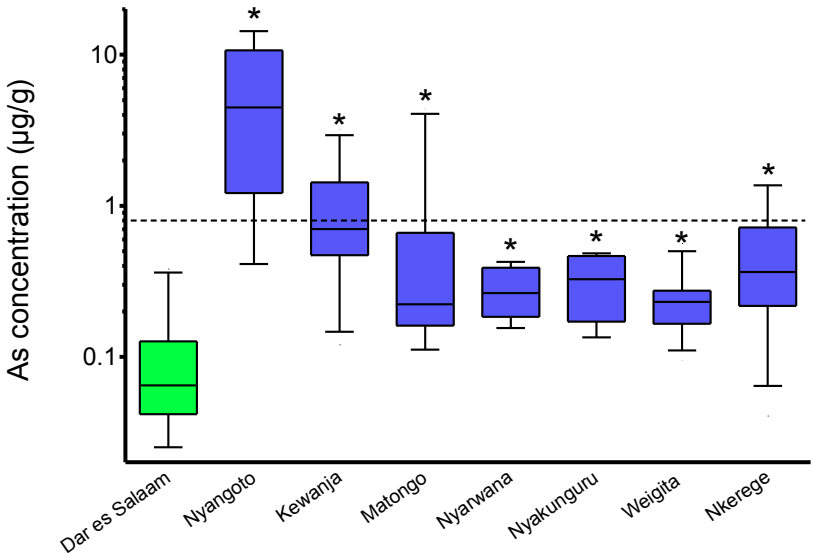


Figure 5. Arsenic concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum. Black stippled lines indicate the levels for which health effects has been indicated in previous studies. *Significantly different from Dar es Salaam ($p<0.05$; Dunnett).

4.2. Antimony (Sb)

The variation in Sb in hair and nails between subjects was comparable for most villages, but higher for Nyarwana in hair and lower for Nyangoto in nails (Figure 6). There were no significant differences in concentrations of Sb in hair from villagers from Tarime District compared to the reference group (ANOVA, DF=7, F=0.52, p=0.8; Figure 6a). There were significantly different Sb-concentrations in nails between subjects from different locations (ANOVA, DF=7, F=2.58, p=0.02). However, no locations had significantly higher Sb-concentrations in subjects compared to the reference group (Dunnett, Figure 6b).

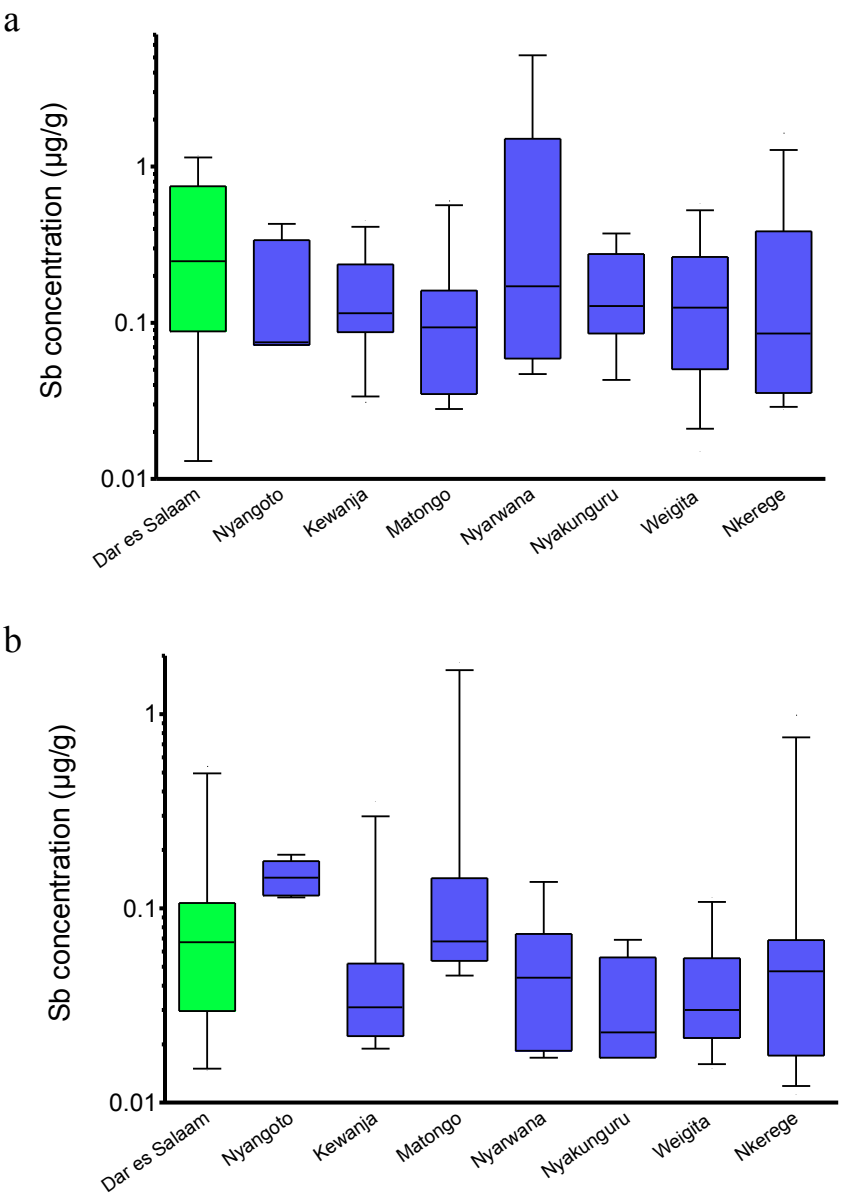
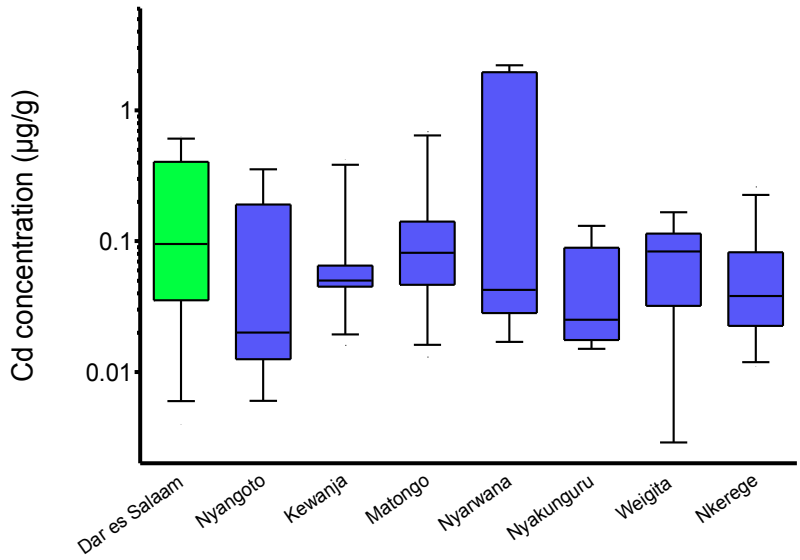


Figure 6. Antimony concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum.

4.3. Cadmium (Cd)

It was particularly high variation in Cd-concentration in hair between subjects for Nyarwana and low in Kewanja (Figure 7a). There were no significant differences in Cd-concentrations in hair between individuals from different locations (ANOVA, DF=7, F=0.99 p=0.4). The variation in nail Cd between subjects were comparable for the locations, and there were no significant differences in nail concentrations between individuals from different locations (ANOVA, DF=7, F=1.36, p=0.2, Figure 7b).

a



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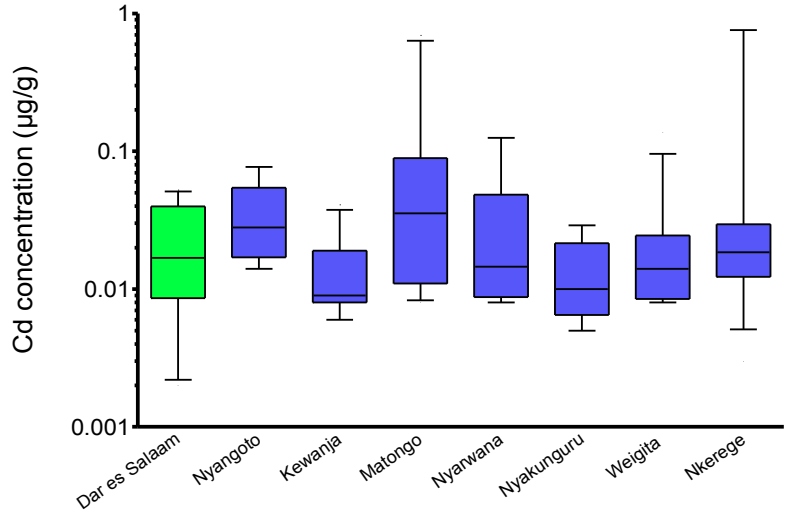

Figure 7. Cadmium concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum.

4.4. Lead (Pb)

The variation in Pb in hair between subjects was higher for Dar es Salaam and Nyangoto, and some villages had lower concentrations in hair than the reference group Dar es Salaam (Figure 8a). There were significant differences in Pb-concentrations in hair between individuals from different locations (ANOVA, DF=7, F=3.76, p=0.002). Villagers from Nyangoto, Weigita and Nkerege had significantly lower Pb-concentrations than the reference group (Dunnett, p=0.01, p=0.0006, p=0.004). The median value in hair ranged from 1.5 (Matongo) to 4.5 (Weigita) times higher in the reference group compared to the median value of subjects from the different villages in Tarime District. The variation in nail concentration was higher for some villages; e.g. Nyangoto (Figure 8b). There were no significant differences in Pb-concentrations in nails of subjects between the locations (ANOVA, DF=7, F=1.04, p=0.4).

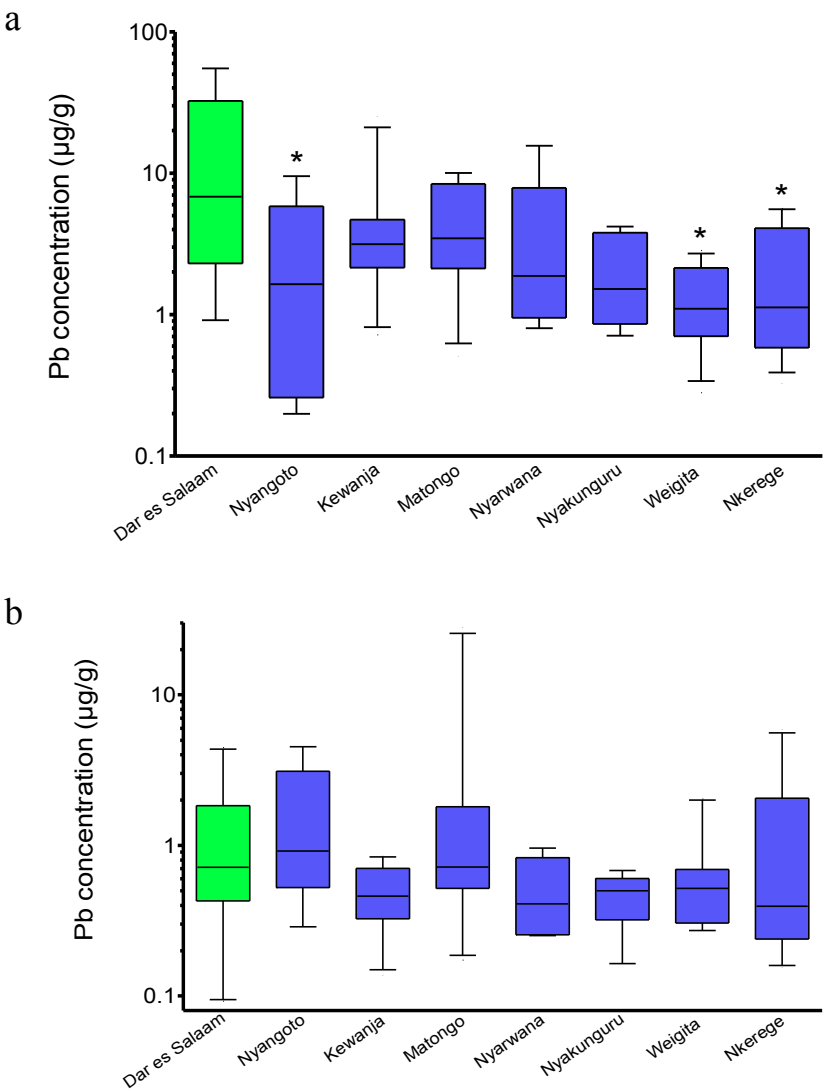

Figure 8. Lead concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum. *Significantly different from Dar es Salaam (p<0.05: Dunnett).

4.5. Zinc (Zn)

It was great difference in the variation in Zn in hair between individuals, and particularly high for Nyarwana (Figure 9a). There were no significant differences in Zn-concentrations in hair between the subjects from the different locations (ANOVA, DF=7, F=1.69, p=0.13). It was great difference in the variation of Zn in nails between individuals, with highest variation in Nyangoto and Matongo (Figure 9b). There were no significant differences in Zn-concentrations in nails between the locations (Kruskal-Wallis, DF=7, p=0.2).

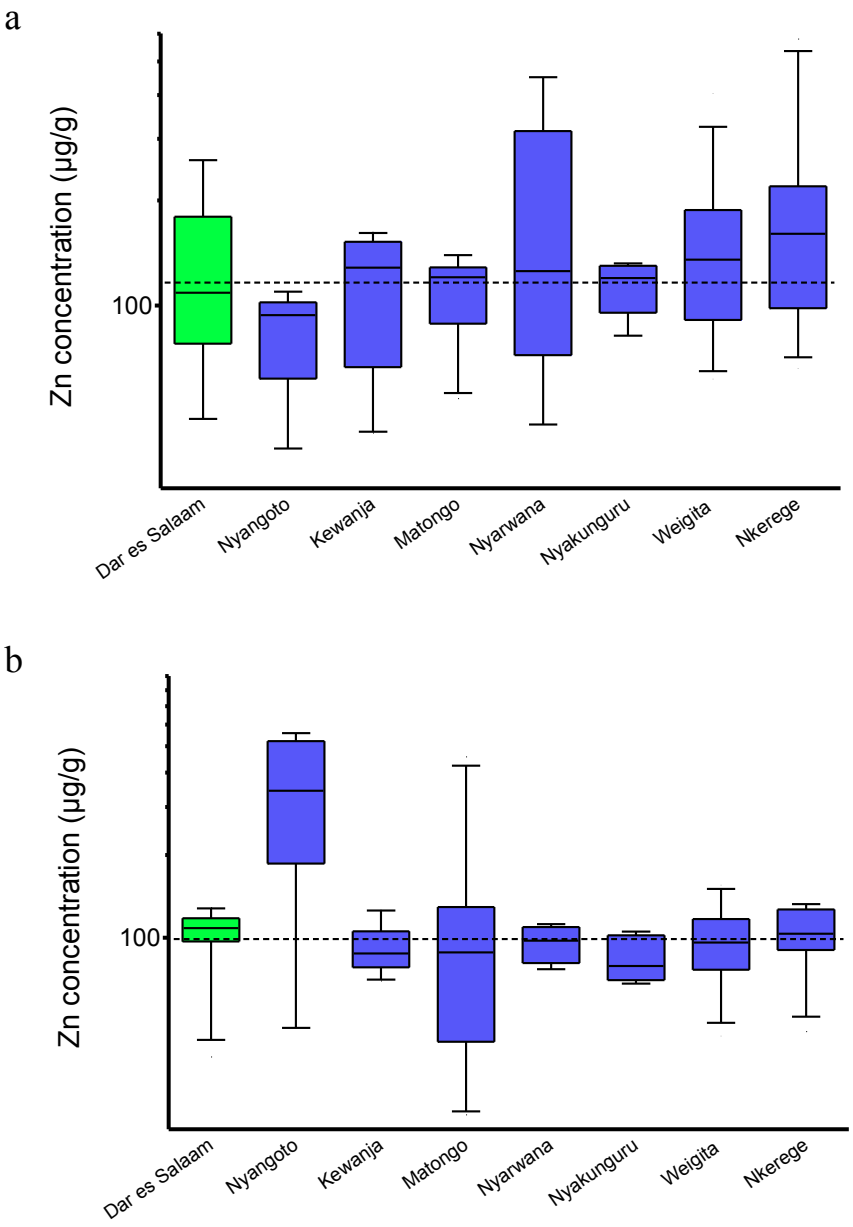

Figure 9. Zinc concentration (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum. The red lines indicates the lowest normal value for Zn in hair (115 µg/g) (Ponzetta et al. 1998) and a value in nails below which subjects had increased cancer risk (97 µg/g) (Rogers et al. 1991).

4.6. Manganese (Mn)

The variation in Mn in hair between subjects was higher for some villages than others, particularly for Dar es Salaam (Figure 10). There were significant differences in Mn-concentrations in hair between the locations (ANOVA, DF=6, F=4.88, p=0.0004; Figure 10a). Subjects from all locations except Nyakunguru had significantly higher Mn-concentration in hair compared to the reference group from Dar es Salaam (Dunnett, p=0.003, p=0.001, p=0.004, p=0.01, p<0.0001). The median hair value of villagers from the different locations in Tarime District ranged from 3.5 (Weigita) to almost 7 (Nkerege) times higher than the median value of Dar es Salaam.

The variation in Mn in nails between subjects was comparable for the different locations, but higher for Dar es Salaam and lower for Nyakunguru (Figure 10b). There were significant different Mn-concentrations in nails between the locations (ANOVA, DF=6, F=3.58, p=0.0044). Inhabitants from all villages except Nyarwana had significantly higher concentrations compared to the reference group (Dunnett, p=0.01, p=0.005, p=0.03, p=0.02, p=0.002). The median nail value of villagers from Tarime District was 4.8 (Matongo) to 6.5 (Nkerege) times higher than the median value of the reference group. The hair and nail samples from Nyangoto are not presented as Mn was not analyzed for at NTNU.

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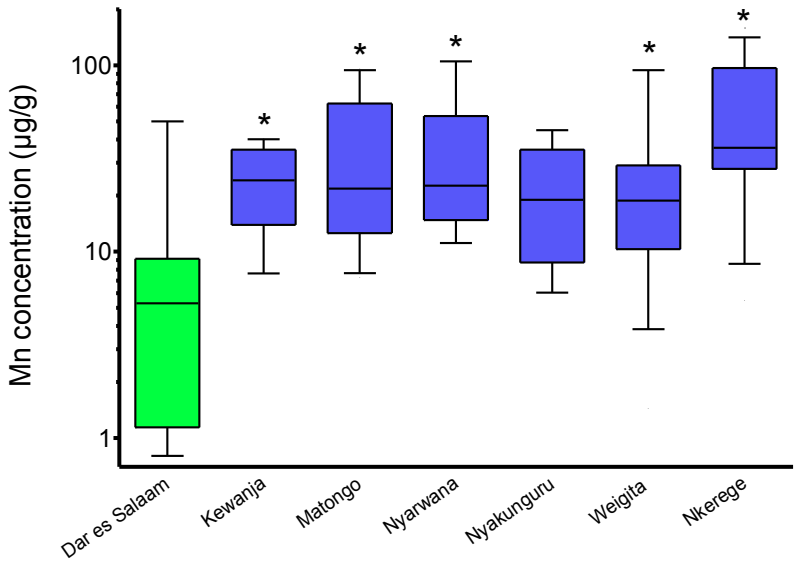

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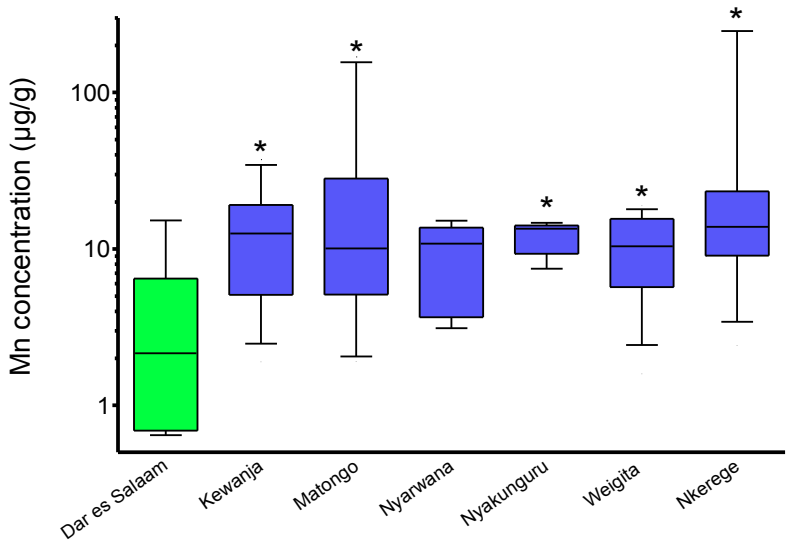

Figure 10. Manganese concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum. *Significantly different from Dar es Salaam ($p<0.05$; Dunnett).

4.7. Copper (Cu)

The variation in Cu in hair and nails between individuals was higher for some villages than for others, and particularly low in nails for Kewanja and Nyakunguru (Figure 11). There were no significant differences in the Cu-concentrations in hair between subjects from the different locations (Kruskal-Wallis, DF=6, p=0.3; Figure 11a). There were no significant differences in Cu-concentrations in nails between villagers in Tarime District and Dar es Salaam (Kruskal-Wallis, DF=6, p=0.12; Figure 11b). The hair and nail samples from Nyangoto are not presented as Cu was not analyzed for at NTNU.

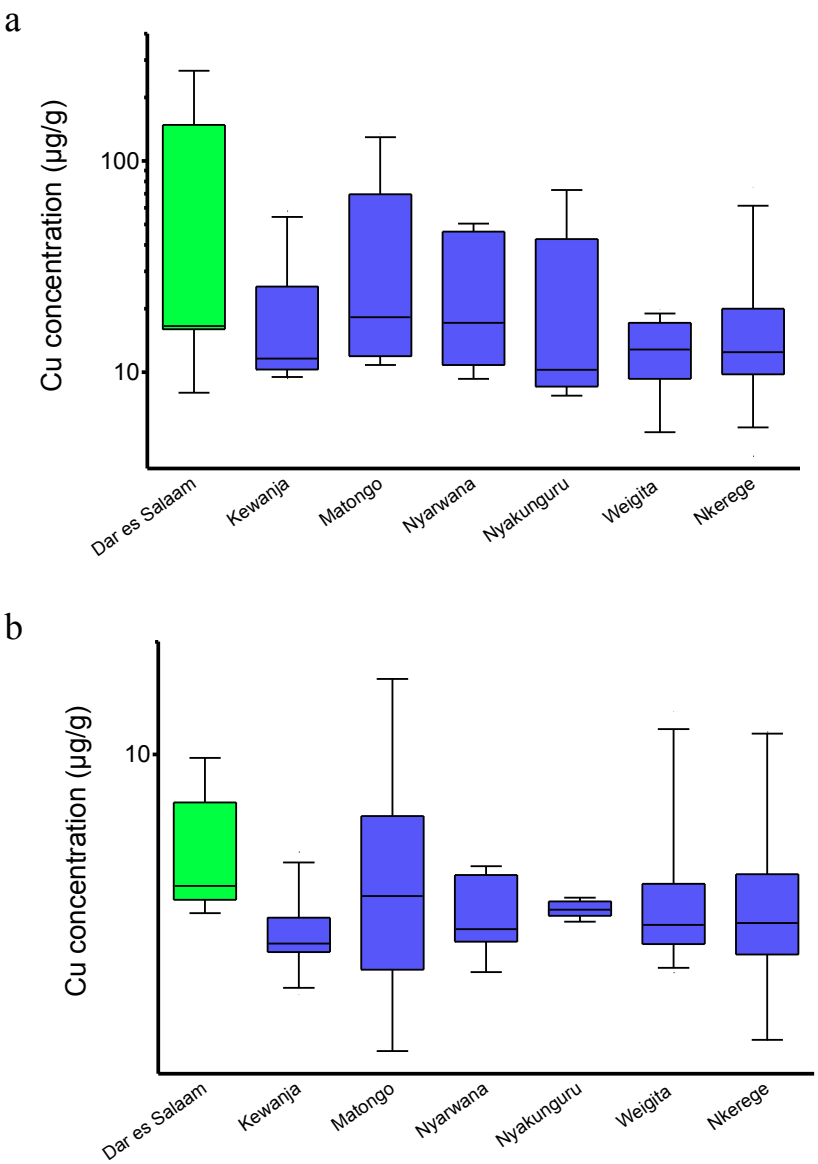

Figure 11. Copper concentration (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum.

4.8. Thorium (Th)

The variation in Th in hair and nails between individuals was higher for some villages than others, and there were no significant differences in Th-concentrations in hair of people from different locations (ANOVA, DF=6, F=1.11, p=0.4, Figure 12a). There were significant differences in Th nail concentration in subjects between the locations (ANOVA, DF=6, F=7.21, p<0.0001). All villagers in Tarime District had significantly higher nail concentrations of Th than the reference group (Dunnett; p<0.0001, p=0.0003, p=0.0001, p=0.0003, p<0.0001, p<0.0001, Figure 12b). The median nail value of villagers from Tarime District ranged from 13 (Matongo) to almost 32 (Nyarwana) times higher than the median for Dar es Salaam. The hair and nail samples from Nyangoto are not presented as Th was not analysed for at NTNU.

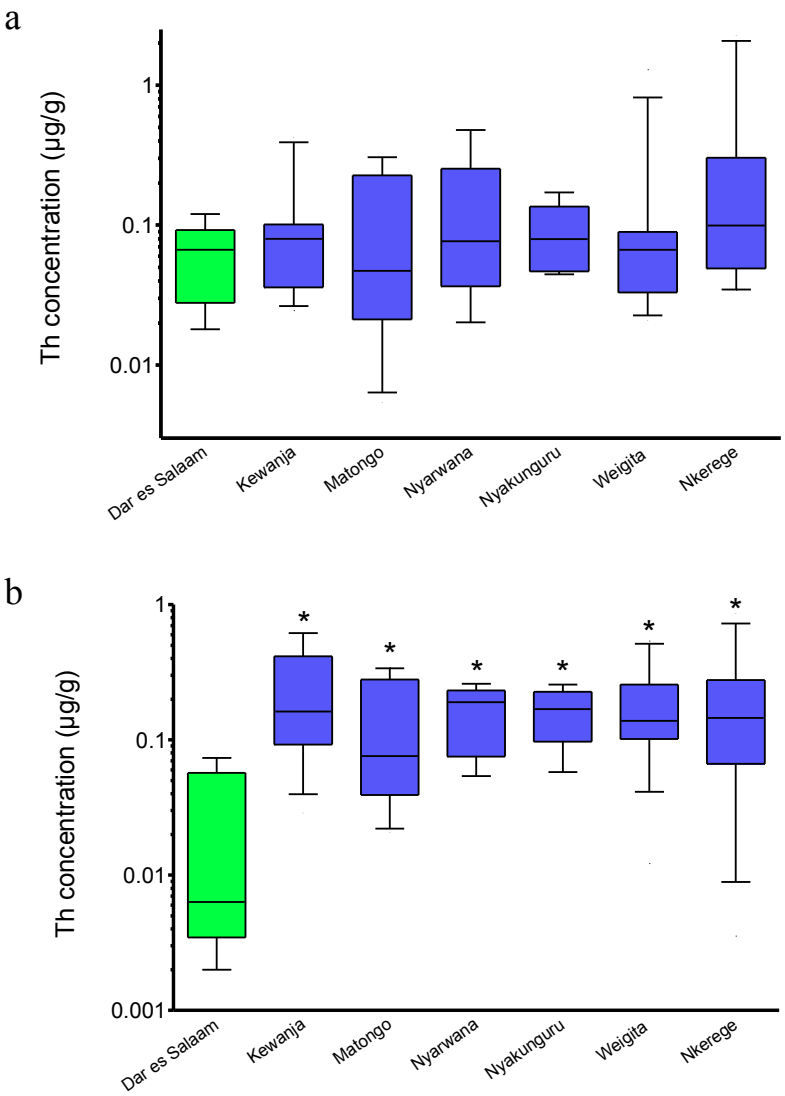

Figure 12. Thorium concentrations (µg/g) in (a) hair and (b) nail samples from all locations; median, quartiles, maximum and minimum. *Significantly different from Dar es Salaam (p<0.05; Dunnett).

4.12 Blood

The variation between subjects in concentration of As in blood was higher for some villages than others, and the median value of subjects from Dar es Salaam were twice as high than the median value of most other locations (Table 7). There were significant differences in As-concentrations in blood between the locations (Kruskal-Wallis, DF=7, p=0.001). Subjects from all locations except Nyangoto and Nyarwana had significantly lower As concentrations than the reference group from Dar es Salaam (Bonferroni-corrected Wilcoxon; Kewanja (p=0.0009), Matongo (p=0.0009), Nyakunguru (p=0.005), Weigita (p=0.001) and Nkerege (p=0.001).

A one-way ANOVA indicated no significant differences in blood concentrations of subjects from different locations for the elements Zn (ANOVA, F=1.50, DF=7, p=0.2) and Mn (ANOVA, F=1.83, DF=7, p=0.1).

Table 7. Blood trace element concentrations (µg/L) of subjects from all locations. Values are presented as median (minimum-maximum). * Significantly different from Dar es Salaam (p<0.05), median in bold.

	As	Sb	Pb	Zn	Mn	Cu
Dar es Salaam	0.40 (0.3-1.5)	1.14 (0.9-2.1)	1.65 (1.3-3.6)	386 (330-600)	0.58 (0.6-0.7)	61 (46-67)
Nyangoto	0.20 (0.2-0.3)	**0.58*** (0.6-0.7)	2.42 (1.7-6.3)	450 (300-520)	0.79 (0.6-1.0)	**67*** (60-84)
Kewanja	**0.20*** (0.2-0.3)	**0.58*** (0.5-1.0)	2.58 (1.5-4.9)	415 (300-580)	0.95 (0.6-2.5)	**74*** (62-89)
Matongo	**0.20*** (0.2-0.3)	**0.60*** (0.5-1.1)	3.14 (1.3-5.5)	422 (290-580)	0.77 (0.5-2.1)	**70*** (56-106)
Nyarwana	0.30 (0.2-0.4)	**0.59*** (0.6-0.7)	1.87 (1.2-4.6)	407 (200-530)	1.07 (0.5-1.3)	**61*** (56-93)
Nuakunguru	**0.20*** (0.2-0.3)	**0.62*** (0.6-0.7)	1.17 (1.1-2.2)	318 (280-350)	0.72 (0.6-1.1)	**73*** (61-87)
Weigita	**0.20*** (0.2-0.4)	**0.61*** (0.6-4.0)	1.37 (0.5-5.5)	395 (270-520)	0.80 (0.5-2.3)	**74*** (58-118)
Nkerege	**0.25*** (0.2-0.3)	**0.60*** (0.6-1.0)	1.22 (0.6-4.1)	399 (280-510)	0.72 (0.5-1.4)	**74*** (64-96)

Blood concentrations were significantly different in subjects from the different locations for the elements Sb (F=5.27, DF=7, p<0.0001), Pb (F=4.64, DF=7, p=0.0003) and Cu (F=3.00, DF=7, p=0.009) in a one-way ANOVA. Subjects from all locations had significantly lower blood concentration of Sb than the reference group from Dar es Salaam (Dunnett; Nyangoto: p=0.001,

Kewanja: p<0.0001, Matongo: p<0.0001, Nyarwana: p=0.0002, Nyakunguru: p=0.0008, Weigita: p=0.0009, Nkerege: p<0.0001). For blood levels of Pb, no differences were found when comparing the reference group with villagers from Tarime District. Finally, subjects from all villages except Nyangoto and Nyarwana had higher blood concentrations of Cu than subjects from Dar es Salaam (Dunnett; Kewanja: p=0.005, Matongo: p=0.01, Nyakunguru: p=0.03, Weigita: p=0.002, Nkerege: p=0.003). No statistical analyses were done for the elements Cd, Mo and Th in blood due to the large proportion of values under LD.

4.9. Factors affecting trace element concentrations

Generalized Linear Model analyses were done for all elements to test for possible influence of gender and age on trace element concentration. Smoking was not tested in the same model as all smokers were men.

4.9.1. Age and gender

Accumulation of As in hair was significantly influenced by both gender (p=0.02) and age (p=0.05, Table 5). The GLM indicated higher As-concentration (µg/g) in men than in women, and decreasing As concentration with increased age. The concentration of the elements Sb, Cd, Zn and Mn in hair was not significantly influenced by gender or age. Gender had a significant influence on the concentration of Pb (p=0.02) and Cu (p=0.003) in hair, but age or smoking did not appear to have an effect for those two metals. There were higher concentrations (µg/g) of Pb and Cu in hair from men compared to women. Age had significant influence on the concentration of Th in hair (p=0.01), with decreasing Th concentration with increased age.

Table 5. GLM with gender and age as explanatory factors for all elements in hair. '**-**' indicate not calculated p-values for individual factors (no significant response in whole model). Significant p-values are shown in bold.

	Whole model	p-values	
	p	Gender	Age
As	0.02	**0.02**	**0.05**
Sb	0.26	-	-
Cd	0.10	-	-
Pb	0.03	**0.02**	0.09
Zn	0.51	-	-
Mn	0.07	-	-
Cu	0.04	**0.003**	0.61
Th	0.01	0.07	**0.01**

Trace element accumulation in nails did not appear to be influence by gender or age: As (p=0.39), Sb (p=0.61), Cd (p=0.53), Pb (p=0.94), Zn (p=0.54), Mn (p=0.54), Cu (p=0.99) and Th (p=0.31). Any differences between subjects appeared to be more strongly affected by the village of habitation than any other factor.

4.9.2. Age and smoking (men only)

GLM analysis was done for male subjects to test for possible interactions between trace element concentration and the response variables age and smoking habits. The responses were not crossed as the dataset was unbalanced, e.g. four of the locations had no smokers. No trace elements in hair of men except As appeared to be significantly influenced by age or smoking: As (p=0.04), Sb (p=0.18), Cd (p=0.93), Pb (p=0.19), Zn (p=0.29), Mn (p=0.09), Cu (p=0.71) and Th (p=0.06). The concentration of As in hair of men was significantly influenced by age (p=0.02) and the GLM indicated decreasing As-concentration (µg/g) with increasing age.

Arsenic was the only trace element in nails for which there appeared to be an effect of age or smoking: As (p=0.03), Sb (p=0.56), Cd (p=0.58), Pb (p=0.24), Zn (p=0.08), Mn (p=0.13), Cu (p=0.11) and Th (p=0.08). The concentration of As in the nails of men was significantly influenced by smoking habits (p=0.02), and the GLM indicated higher As concentration (µg/g) in smokers compared to non-smokers.

4.10. Relative trace element composition

Principal component analysis (PCA) was used to evaluate the relationships for all of the eight elements in hair and nail (Figure 13). The PCA for hair samples resulted in a model for which 47% of the variability was included in the first principle component (Prin1) and 19% was included in the second principle component (Prin2, Figure 13a). In the Eigenvalue analysis of nail samples, 36% and 28% of variability were included in the first and second principle components, respectively (Prin1 and Prin 2, Figure 13b). The concentrations are similar when they are close together in the plot. Consequently, the profile of subjects from Dar es Salaam for both hair and nail concentrations appear to be outside the profile of subjects from Tarime District some extent, but there was a clear overlap.

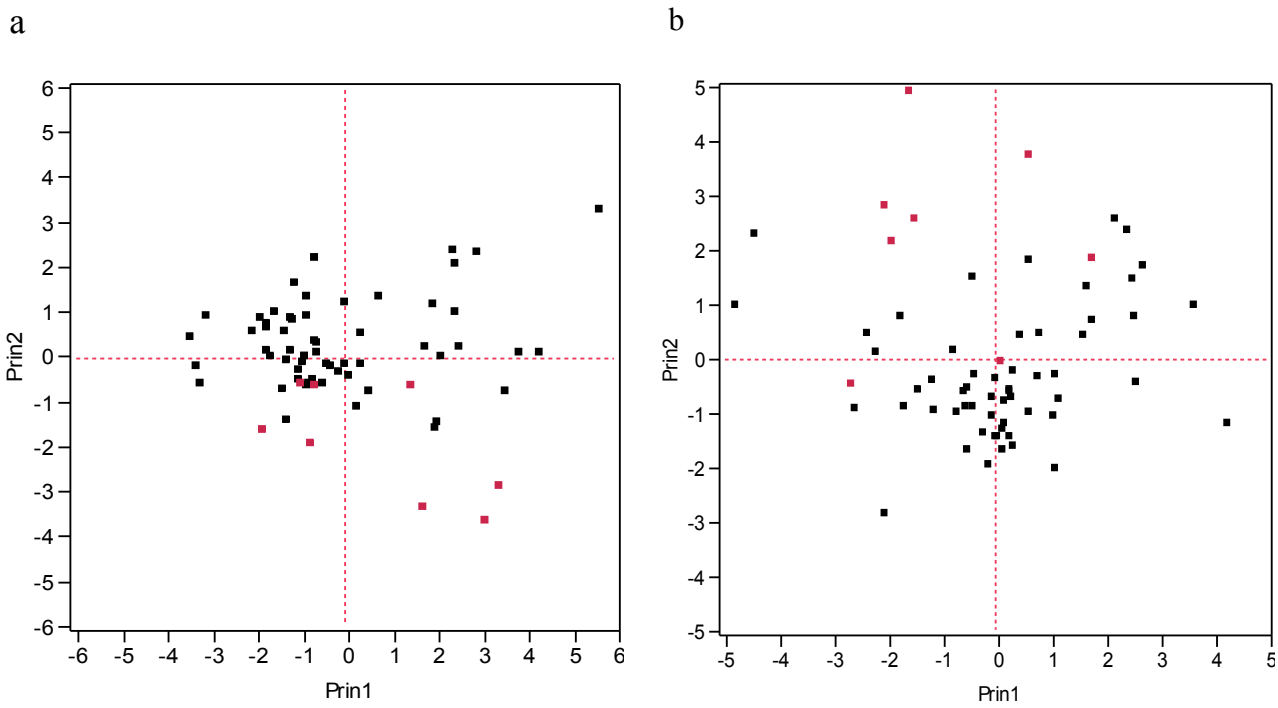

Figure 13. Principal component analysis for all elements in (a) hair and (b) nails. Red squares are values from the reference group Dar es Salaam.

4.11. Relationship between hair and nails

Half of the trace elements had significant correlation between matched hair and nail samples in subjects from the seven investigated villages, excluding the reference group (Table 6 and Figure 14). It was a significant correlation between hair and nail concentrations for the elements As ($p=0.0002$), Cd ($p=0.03$), Zn ($p<0.0001$) and Th ($p=0.04$). The remaining elements had no significant correlation between matched hair and nails samples. Some numbers of observations (N) are missing because five nail samples from Nyangoto were analyzed at NTNU, and Mn, Cu and Th were not analysed for at NTNU. Additionally, one observation was excluded for Cd as the hair value had concentrations <LD as well as low tissue in-weight.

Table 6: Linear regression for hair and nail concentrations for all elements. Number of observations (N), adjusted R^2, F-value and p-value are presented. Significant p-values are shown in bold ($p<0.05$).

	N	R^2	F-value	p-value
Log As	62	0.20	15.6	**0.0002**
Log Sb	62	0.01	0.69	0.41
Log Cd	61	0.18	1.83	**0.03**
Log Pb	62	0.01	0.88	0.35
Log Zn	62	0.98	3788	**<0.0001**
Log Mn	57	0.01	0.68	0.41
Log Cu	57	0.04	2.34	0.13
Log Th	57	0.07	4.55	**0.04**

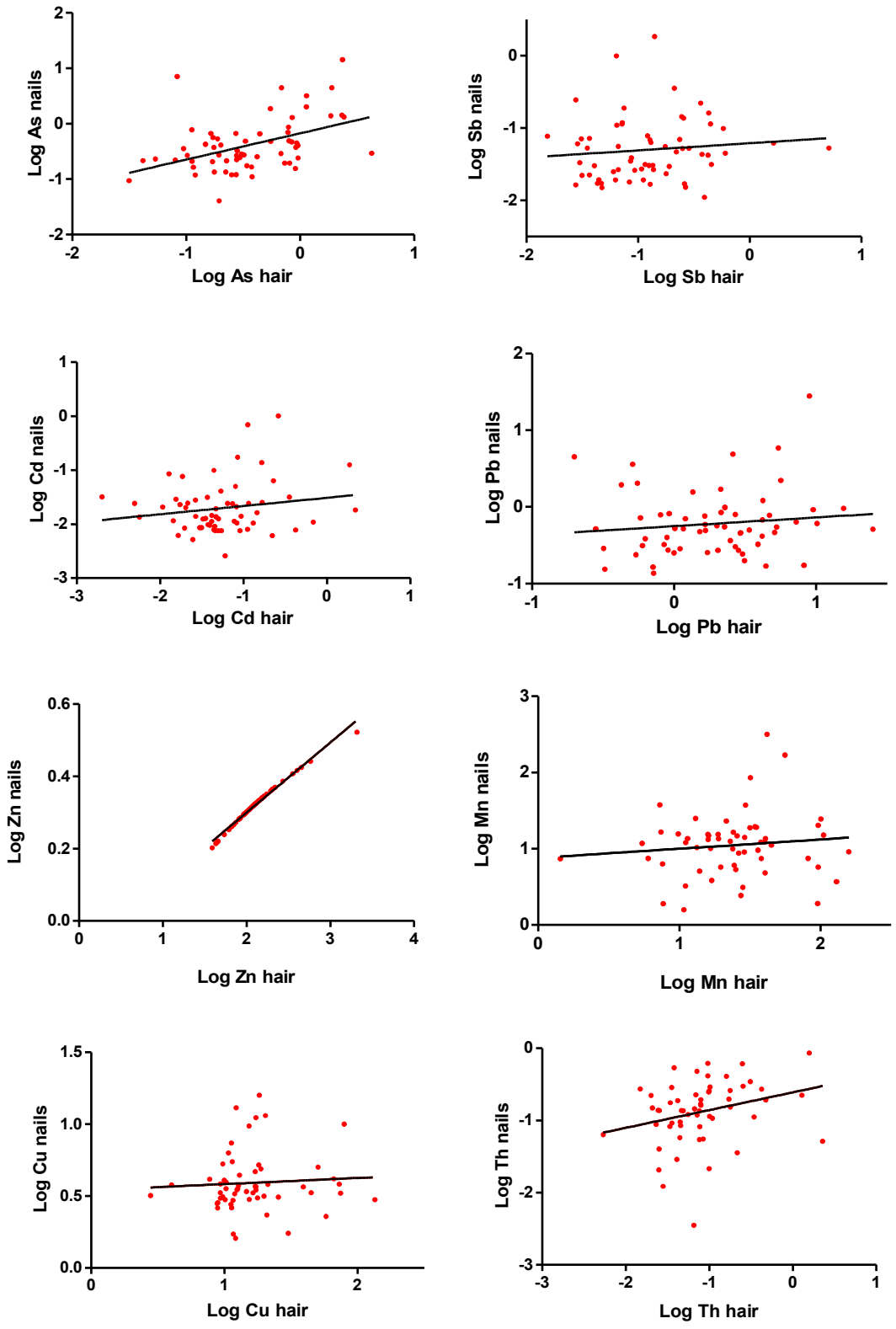

Figure 14. Relationship between hair and nail samples with linear regression (see Table 6 for regression results).

5. Discussion

5.1 Arsenic

5.1.1 Hair

Hair concentration of As in villagers from Kewanja was significantly higher than the reference group from Dar es Salaam. The median concentration of the subjects from Tarime District was higher than As concentration in hair from unexposed populations in other studies, whereas the median for the reference group Dar es Salaam was comparable with other non-exposed populations (Rodushkin and Axelsson 2000; Chojnacka et al. 2006; Gellein et al. 2008).

The normal range of As concentration in human hair has been reported to range from 0.02 to 1.0 µg/g, and it has been suggested that normal As levels are < 1.0 µg/g in healthy individuals (Chiou et al. 1997; Hindmarsh 2002). The International Conference on Arsenic Exposure and Health Effects proposed an upper limit for As in hair of 0.8 µg/g (Mazumder 2003). Close to 30% of villagers from the Tarime district had values equal to or above this limit, whereas none of the subjects from Dar es Salaam exceeded this value. Four out of five (80%) of the children (<10 years) had As hair concentrations ≥0.8 µg/g, and more than 50% of the villagers in Kewanja had hair arsenic concentrations above this limit.

In contrast, the US National Research Council concluded that non-exposed populations would have As-concentration in hair ranging from 0.02 to 0.2 µg/g based on 12 scientific studies (NRC 1999). If the upper limit of normal concentration is set to 0.2 µg/g, the findings of the current study are of greater concern since the median values for all villages except Nkerege and Weigita were higher. All hair samples from Kewanja had higher concentrations of As than 0.2 µg/g, and Nkerege and Weigita were the only villages in Tarime District with more than half of the values below this limit. Overall, two-thirds of all hair samples from Tarime District had higher As concentrations than 0.2 µg/g. Some subjects from Dar es Salaam had higher As concentration than this limit, but there was large variability in this group. A possible explanation could be related to the finding of the dramatic increase of sediment-metal levels in the direct vicinity of Dar es Salaam over the last decade (De Wolf et al. 2001). Domestic and industrial wastes are discharged to aquatic environment without prior treatment, and there are high concentrations of trace elements such as As, Cd, Cu, Mn, Pb and Zn in the wastes being released (Muzuka 2007). These finding may partly explain the elevated As values in some subjects from Dar es Salaam compared to normal range. Fish and shellfish are sources of mainly organic As (Cleland et al. 2009) and is a

minor source of As in hair as nearly 95% of the As incorporated in hair are estimated to be inorganic species (Orloff et al. 2009). Electromyographic abnormalities was reported in half of the subjects with As hair concentration >1 µg/g in a study of people exposed to As in drinking water (Hindmarsh et al. 1977). Electromyographic abnormalities include decreased nerve conduction amplitude with little change in nerve conduction velocity (Fowler et al. 2007). More than 10% of villagers from Tarime District exceeded hair As concentration of 1 µg/g, but none of the subjects from Dar es Salaam.

There are some reasons why As may be more variable in hair compared to nails, including possible absorption from water (Orloff et al. 2009) or removal during washing (NRC 1999). It has however been indicated that washing procedure would not essentially influence the concentration because of the strong complex of As with sulphide groups in hair (Samanta et al. 2004). Arsenic concentration in human hair from As affected areas in previous studies have similar or higher concentrations than the villagers from Tarime District (Chiou et al. 1997; Mandal et al. 2004; Rapant et al. 2006). The current study is therefore consistent with previous finding of exposed populations, e.g. as a result of As-exposure in mining areas.

5.1.2 Nails

Nail arsenic concentration of the reference group Dar es Salaam was significantly lower than the concentration of subjects from all other locations in the current study. The concentration of As varied between subjects within each village and between villages. Villagers from Tarime District had higher median concentration than found previously in unexposed populations (Chaudhary et al. 1995; Mandal et al. 2003).

Different normal ranges or guidelines have been proposeed for As in nails. Normal concentration has been suggested to range from 0.02 to 0.5 µg/g (Narang et al. 1987; Takagi et al. 1988). Almost 20 villagers (30%) from Tarime District had concentrations in nails exceeding the suggested normal range. Studies from As-contaminated areas have found similar or higher nail concentrations of As compared to the current study (Mandal et al. 2004; Rapant et al. 2006). All subjects from the reference group in Dar es Salaam had As concentration within the normal range.

In contrast to most other studies, Mazumder (2003) suggested an upper normal level for As concentration in nails to be 1.3 µg/g. There are various possible reasons for the wide concentration range between different studies, including lack of standardized washing procedures and the potential for external contamination (Orloff et al. 2009).

It is well established that some elements, such as arsenic, will accumulate in hair and nails following exposure through diet or drinking water (Chiou et al. 1997; Gellein et al. 2008). Several studies have found a positive correlation between As in drinking water and concentration in hair/nails (Biswas et al. 1998; Mandal et al. 2003; Samanta et al. 2004). Elevated levels of As were detected in water from Tarime District compared to control sites in the study of Almås et al. (2009). The concentration of As in rivers and ponds were found to be higher than the accepted standards of WHO drinking water guidelines of 10 µg/L, and some locations had As levels above 50 µg/L (Table 1). Subjects drinking water with As concentration exceeding 50 µg/L have previously been found to acquire elevated As hair concentration above 0.4 µg/g (Goldsmith et al. 1972). The finding is supported by another study in which As concentration in groundwater samples exceeded the WHO drinking water guidelines, and a positive correlation was observed between As levels in groundwater and human hair samples (Agusa et al. 2006). Groundwater has been found to exceed the WHO's guidelines for As (10 µg/L) in countries from every continent (Kapaj et al. 2006). Residents from Bangladesh provide the most well-known example of arsenicosis in a population. An estimate suggested that 35-77 million people were exposed to inorganic As, and two thirds of the tube wells contained As concentrations exceeding the WHO drinking water guidelines (Biswas et al. 1998; Nickson et al. 1998; Smith et al. 2000).

Exposure to As may cause DNA damage through mutations, alter DNA repair, cause oxidative stress, and induce chromosome abnormalities and cell proliferation (Roy and Saha 2002; Russi et al. 2005). The risk for skin and bladder cancer was reported to increase twofold for subjects with As nail concentrations exceeding 0.8 µg/g (Karagas et al. 2001; Karagas et al. 2004), well within the range suggested by Mazumder (2003). Almost 15 individuals (20%) from Tarime District had As concentration in nails above this level, but none from Dar es Salaam. Villagers in Nyangoto could particularly be at risk as all subjects except one had As concentrations in nails above 0.8 µg/g. Various skin lesions were observed in some of the subjects of the present study (Appendix 5), but the prevalence was not quantified. It has been found that As levels were high in hair and nail tissue of individuals with As-associated skin lesions (Kapaj et al. 2006). Skin lesions, hypo- and hyperpigmentation, and hard patches of skin on palms and soles of hands and feet can be caused by arsenicosis. The symptoms of arsenicosis are assumed to appear within 8-10 years of consumption of water with elevated As concentrations (Phan et al. 2010). The use of contaminated water for drinking and domestic purposes may explain the skin lesions observed in subjects from Tarime District. Coal rich in As is a potential source of inorganic As exposure as it is commonly used for heating and cooking in Africa (Shraim 2003). Women normally do all the cooking in Tanzania (Wandel and Holmboeottesen 1992), but men were found to have higher As

concentration in hair than women in the current study. Potential exposure to inorganic As in coal does consequently not appear to be an important factor for the results found in the current study.

The results from this study has indicated that some individuals from the Tarime District have been exposed to As at concentrations much higher than levels that would lead to background concentrations in hair or nails. The difference were most clearly observed for nails, but apparent for both tissues. Values of As were generally higher than concentrations found in non-exposed subjects from other studies. About 30% and 20% of the individuals from Tarime District had higher concentrations than suggested upper normal level of As in hair and nail respectively.

5.2. Other elements

5.2.1 Antimony (Sb)

No significant differences in Sb concentrations were found between Tarime District and Dar es Salaam for neither hair nor nail samples. It is not known whether effluents from North Mara Gold Mine contain Sb. Elevated levels of Sb have previously been detected in regions with long history of intensive ore mining, and high concentration was also found in human tissue from residents in the mining areas (Rapant et al. 2006; Liu et al. 2011a). Studies of other non-exposed populations have found similar levels of Sb in both hair and nail samples compared to the current study (Katayama and Ishida 1987; Chaudhary et al. 1995). Initial clearance of Sb is rapid in humans, and more than 90% of an intravenous dose will be found in the urine within 24 hours (Tylenda and Fowler 2007). It would therefore have been challenging to detect Sb in human tissues as a result of possible low-level chronic exposure even if the element was present in Tarime District.

5.2.2 Cadmium (Cd)

The levels of Cd in hair and nail samples were not significantly different between subjects from Tarime District and the reference group Dar es Salaam. Similar Cd concentrations have been found in previous studies of populations with normal Cd values in both hair and nails as subjects from the current study (Rodushkin and Axelsson 2000; Gellein et al. 2008). Results from studies of exposed populations have found higher concentrations of Cd compared to subjects in the current study including the reference group (Agusa et al. 2006; Were et al. 2008). The low Cd concentration in human samples indicated that villagers from Tarime District were not exposed to higher levels of Cd than normal background.

5.2.3. Lead (Pb)

Pb concentration in hair was significantly lower in subjects from Tarime District than in the reference group Dar es Salaam, but no differences were found in the concentration of nail samples. The median concentration of villagers from Tarime District were comparable to previous studies of non-exposed populations, but the median value of subjects from Dar es Salaam was higher (Rodushkin and Axelsson 2000; Chojnacka et al. 2006; Gellein et al. 2008). Pb contaminated street dust, residential soil and lead-based paint from the buildings in Dar es Salaam may have contributed to the elevated value in hair of the reference group (Asante et al. 2007). Another possible source of exposure is lead added to gasoline. The Pb concentration in gasoline from Tanzania was estimated to be 0.5 g/L in year 2000, resulting in a total Pb release through gasoline of 100 tonnes per year (Hodes et al. 2003). However, the government of Tanzania developed a leaded gas phase-out action, and today there is no known use of leaded gasoline in the country (WHO 2009). Other sources could explain the higher Pb concentraion in hair of subjects from Dar es Salaam, e.g. lead-based paint.

5.2.4 Zinc (Zn)

No significant differences were found in Zn concentration between villagers from Tarime District and the reference group for neither hair nor nails. The results indicate that subjects from Tarime District were not exposed to Zn despite the high concentrations of Zn in the spill from North Mara Gold Mine (Table 1). Increased Zn intake was correlated with increased Zn concentration in hair in an epidemiological study (Pekarek et al. 1979). The normal value for Zn in hair have been suggested to be within a minimum range of 115-160 µg/g and a maximum range of 190-210 µg/g (Ponzetta et al. 1998). More than one third (37%) of the all subjects in the current study had hair Zn concentrations below the minimum normal value. The median value for villagers from Nyangoto and Dar es Salaam was less than 115 µg/g. On the other hand, more than five subjects (4.5%) in the study had hair Zn concentration exceeding the maximum normal value. The remaining subjects from Tarime district and Dar es Salaam had Zn hair values within the normal range, and the concentrations were comparable to unexposed populations from other studies (Pan et al. 1993; Rodushkin and Axelsson 2000).

In addition to low Zn hair concentration, a tendency towards low Zn nail levels was observed in the current study. It has been indicated that Zn nail values below 97 µg/g may be associated with increased risk for oral cancer, with an estimated 1.6 times higher risk of oral cancer (Rogers et al. 1991). Almost half (49%) of the subjects from current study had nail Zn concentration below 97 µg/g. Villagers from Kewanja, Matongo, Nyarwana and Nyakunguru had Zn median value below

this limit, although the groups were not significantly different. Studies of populations with normal range of Zn levels in nails were higher than concentrations of subjects from the current study (Oluwole et al. 1994; Chaudhary et al. 1995).

Some trace elements have been involved in describing the prevalence of prostate cancer, which is the second leading cause of cancer mortality in men (Tan and Chen 2011). The study of Tan and Chen (2011) found lower concentration of Zn in hair for the prostate cancer group compared to the healthy individuals (Figure 15). The peak of Zn concentration in hair for cancer group was approximately 150 µg/g, and 225 µg/g for healthy group. The median Zn concentration in hair of subjects from the current study appeared to be lower than the median value of both healthy and cancer group, but the ranges were comparable.

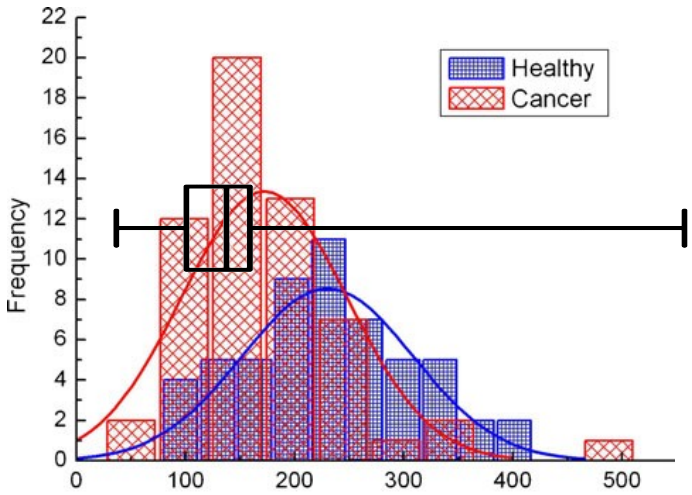

Figure 15. The frequency histogram and corresponding estimated probability distribution of Zn concentration (µg/g) in hair for prostate cancer group (red line) and healthy group (blue line): modified from Tan and Chen (2011). The horizontal box plot represents Zn hair values from all male subjects of the current study; median, quartiles, minimum and maximum.

Low levels of Zn in hair and nails may aggravate the arsenic toxicity (Samanta et al. 2004), and a review of epidemiological studies reported that Zn promotes the repair of tissues damaged by As (Engel et al. 1994). The low concentrations of Zn in subjects from Tarime District may have caused increased toxicity of As.

The findings of the current study appear reasonable as the risk of inadequate Zn intake has been estimated to be 28% in the population from Sub-Saharan Africa (Wuehler et al. 2005). The proportion of Zn from animal sources is lower in Africa compared to other regions, but the proportion from wheat is higher. A study found that increasing concentration of Pb may decrease concentrations of Zn in humans (Nowak and Chmielnicka 2000). This might partly explain the low Zn levels of the reference group in addition to dietary habits.

There were no differences between villagers of Tarime District and subjects in the reference group from Dar es Salaam, but the low Zn concentrations in hair and nails indicate that some subjects may suffer health impairment associated with Zn deficiency. The low level of Zn was presumably due to factors such as dietary habits and was not related to the mining activity in North Mara.

5.2.5 Manganese (Mn)

Subjects from all locations except Nykunguru had significantly higher concentrations of Mn in hair compared to the reference group from Dar es Salaam. The hair levels of Mn in villagers from Tarime District was higher than normal ranges from previous studies (Chojnacka et al. 2006; Saric and Lucchini 2007) and comparable with studies of Mn exposed populations (Samanta et al. 2004; Agusa et al. 2006). The reference group from Dar es Salaam had concentrations in hair within normal range. Inhabitants from all villages except Nyarwana had significantly higher Mn concentrations in nails than subjects from Dar es Salaam. Villagers from Tarime District had higher Mn concentration in nail samples than a non-exposed population from Sweden (Rodushkin and Axelsson 2000), but the subjects from Dar es Salaam were found to be within the normal range.

Elevated Mn levels in hair was found to be inversely related to general intelligence scores in a study investigating the association between Mn level in hair and neuropsychological function and behavior in children (Wright et al. 2006). Mn is a known mutagen (Beckman et al. 1985), and studies on environmental exposure of Mn suggest that the element is a neurodevelopmental toxicant (Takser et al. 2003; Crinella 2006). Significant interactions have been found between the elements As and Mn, but potential health effects of high Mn concentrations on As toxicity are not well documented (Layrargues et al. 1998; Samanta et al. 2004). Recommended guideline value for Mn in drinking water is 0.4 mg/L (WHO 2008). The Mn concentration has not been investigated in water in the vicinity of the North Mara Gold Mine. It is therefore important to examine the water in Tarime District to clarify whether concentration of Mn exceeds the WHO drinking water guideline value.

Subjects from some villages in Tarime District had significantly higher Mn concentrations than the reference group in both hair and nails, and the concentrations in the villagers were above the normal range for Mn. Observed concentrations of Mn in hair and/or nails have been associated with health impairments in previous studies.

5.2.6. Copper (Cu)

There were no significant difference in Cu concentrations in hair and nails between subjects from Tarime District and Dar es Salaam. It is not straightforward to determine if the hair concentrations were elevated or not as there are large variations in Cu hair values reported from previous studies (Pasha et al. 2010; Massadeh et al. 2011). Subjects from both Tarime District and Dar es Salaam had a median value within the concentration range from unexposed populations (Gellein et al. 2008; Mohammed and Spyrou 2008), as well as residents from exposed areas (Samanta et al. 2004; Agusa et al. 2006). All nail samples from Tarime District and Dar es Salaam had lower concentrations compared to populations in other studies (Rodushkin and Axelsson 2000; Samanta et al. 2004).

The concentration of Cu in human samples indicates that villagers from Tarime District were not exposed to Cu, and the conclusion is supported by comparisons with previous studies of other unexposed populations.

5.2.7. Thorium (Th)

There were no significant differences in Th-concentrations in hair between villagers from Tarime District and the reference group. Subjects from both Tarime District and Dar es Salaam had higher Th concentration in hair than found in previous studies of unexposed populations (Jaiswal et al. 1985; Rodushkin and Axelsson 2000). All villagers from Tarime District had significantly higher nail concentrations of Th than the reference group from Dar es Salaam. A study of an unexposed population has reported Th concentration in nails similar to the reference group (Rodushkin and Axelsson 2000), but the villagers from Tarime District had much higher Th concentrations in nails.

Estimation of Th in human tissue is difficult, primarily because it is present at extremely low concentrations (Jaiswal et al. 1985). Previous studies have found that background levels of Th vary significantly between people, and the concentration may change for the same individual within a day (Mohagheghi et al. 2005). There were variations in Th concentration between subjects in hair and nails in the current study. Most hair values of people from Dar es Salaam were below LD for Th, which might explain the difference between hair and nail concentrations of the reference group and villagers from Tarime District.

Industrial and mining exposure to Th has resulted in an increased incidence of chronic respiratory diseases, liver damage, and lung-, pancreal-, and colorectal cancer (Meyer et al. 1979; Polednak et al. 1983; Najem and Voyce 1990). It was estimated 1.7 times higher risk of lung cancer and four

times higher risk of pancreatic cancer (Polednak et al. 1983). The elevated level was suggested to be primarily due to ingestion and inhalation of Th in the area surrounding the mines (Meyer et al. 1979).

Concentrations of Th in nails were significantly higher in subjects from Tarime District than in the reference group, but no difference was detected for hair samples. Both hair and nail samples from Tarime District had higher concentrations than found in previous studies of Th in human tissue.

5.3. Blood

5.3.1. Arsenic

Subjects from all locations except Nyangoto and Nyarwana had significantly lower As concentrations than the reference group from Dar es Salaam. Normal blood levels of As in blood are reported to range from 0.3 to 2 µg/L in populations with no unusual exposure (NRC 1999). None of the subjects from the current study had As blood levels above the suggested normal range. Exposure to As through seafood is a probable explanation for the higher As levels in the reference group than found in the the villagers from Tarime District (Samanta et al. 2004). Organic As concentration is high in fish and marine crustacea (Cullen and Reimer 1989), and As intake via seafood consumption has been found to be low in most inland areas of Africa (Banza et al. 2009). A study from Norway found higher As blood concentrations in people living near the coast compared to the subjects living inland (Blekastad et al. 1984). Correspondingly, citizens of Dar es Salaam would be expected to have higher organic As exposure due to higher seafood consumption along the coast of the Indian Ocean than the villagers in Tarime district, located inland.

The levels of As in blood were low for both villagers in Tarime District and Dar es Salaam compared to reported levels from previous studies of As exposed populations (Fowler et al. 2007). The higher As levels in subjects from Dar es Salaam were expected to mainly consist of organic As species, which would not be of great concern as only intake of inorganic As has been related to adverse health effects.

5.3.2. Other trace elements

There were no significant differences in blood concentrations of Zn between subjects from Tarime District and the reference group. The empirical lower normal limit for Zn in fasting blood is 700 µg/L (Pilch and Senti 1985). The median values of Zn were below the suggested lower level of subjects from all locations in the current study. However, Zn in blood is not a sensitive indicator

for Zn status, thus not reflecting potential risk of Zn deficiency (Pekarek et al. 1979). Several factors may affect Zn blood concentration, including higher concentration after fasting, lower levels after a meal, and individual factors such as gender and age (Hotz et al. 2003).

No significant differences were found for the elements Pb and Mn in blood between villagers from Tarime District and the reference group. Concentrations were low in subjects of the current study compared to normal levels of Pb and Mn in blood from other studies (Chiba and Masironi 1992; Saric and Lucchini 2007). Individuals from all locations had significantly higher blood concentration of Sb than the reference group, but the values were lower than exposed populations from elsewhere (Tylenda and Fowler 2007). Subjects from all villages except Nyangoto and Nyarwana had higher blood concentrations of Cu than in the reference group, but all values were low compared to previous findings (Ellingsen et al. 2007).

Blood concentrations of some trace elements were significant different between subjects from Tarime District and Dar es Salaam, but the median value was low for most elements. The blood concentration was generally lower in the current study compared to other exposed and unexposed populations. Care should be taken with the current results of blood as the concentrations were lower than the samples analyzed at NTNU.

5.4. Differences between locations

There were lower As concentration in the reference group for hair and nails, but the blood concentrations were higher in the villagers from Tarime District (Table 8). Subjects from Dar es Salaam were found at lower levels for Mn in hair, Mn and Th in nails, and Cu in blood compared to villagers from some or all locations in Tarime District. People from Tarime District had lower concentration of the elements Pb in hair and Sb in blood. There were no differences between the locations for the remaining trace elements in hair, nails and blood.

Table 8. Results of statistical analyses of trace elements in different matrices. **DL**: significantly lower values in the reference group (Dar es Salaam) than for some or all other locations. **DH**: significantly higher values in the reference group than some or all other locations. No significant differences: **n.s.** Grey boxes: no statistical analysis as >20% of the concentration values were below LD.

	As	**Sb**	**Cd**	**Pb**	**Zn**	**Mn**	**Cu**	**Th**
Hair	DL	n.s.	n.s.	DH	n.s.	DL	n.s.	n.s.
Nail	DL	n.s.	n.s.	n.s.	n.s.	DL	n.s.	DL
Blood	DH	DH		n.s.	n.s.	n.s.	DL	

The profile for Dar es Salaam subjects for hair and nail concentrations appear to group to some extent separate from values for Tarime villagers in the principal component analysis, but the reference group was not tightly clustered. The full picture of all trace elements in subjects from all locations appear to have small differences as there are some overlap between the profile of Dar es Salaam and the other villages.

5.5. Factors affecting trace element concentrations

Concentrations of As in hair were influenced by gender, and levels of As were higher in men than in women. Other studies have found similar results, with significantly higher As concentration in hair from men (Chiou et al. 1997; Mandal et al. 2003; Wright et al. 2006). Conversely, some studies found no gender differences for As concentration in hair (Asante et al. 2007; Mao et al. 2010; Phan et al. 2010), and As concentration in hair was not different between genders in exposed populations in China (Shraim et al. 2003; Liu et al. 2011a). The current study found no effect of gender with respect to As in nails. In accordance with this result, an investigation of gender and concentration in fingernails found no correlation between As levels and gender (Chaudhary et al. 1995).

Trace element accumulation in hair and nails was not influenced by gender for the elements Sb, Cd, Zn and Mn. Gender had influence on the concentration of Pb and Cu in hair, and men had higher levels of Pb and Cu than women. Other studies found no difference for Sb concentration between male and female hair in subjects from the same area (Liu et al. 2011a), and Pb concentrations was higher in men (Nowak 1998). Both Cu and Pb were higher in males in the study of Chojnacka et al. (2006), but hair levels of Mn and Cd had no significant relation to gender (Chojnacka et al. 2006; Wright et al. 2006). Zn concentration in nails was found to be higher in female subjects than in males (Chaudhary et al. 1995).

The results from different studies appear contradictory. Some studies have found higher concentrations of Cu and Mn in hair from females compared to males (Rodushkin and Axelsson 2000), whereas concentrations of Cd and Zn were found to be significantly higher in males in the study of Nowak (1998). Boys appeared to have higher hair levels for all non-essential elements while girls had higher levels of Zn in a study of children living in the vicinity of a mining area (Laura Barbieri et al. 2011). The hair concentrations of Cd and Mn were not significantly related to gender in the study of Wright et al. (2006).

Concentration of As in hair were influenced by age in the current study, with decreasing As concentration with increased age. No correlation was found between As levels and age in nails. Other studies have found higher As levels in children than in other age groups (Armienta et al. 1997; Liu et al. 2011a). The cause may be due to children's higher food and water consumption relative to their body weight and their exploration of the environment by hand and mouth (Banza et al. 2009). Accumulation of As is related to metabolism, and children and elderly people may therefore accumulate more As than other age groups (Liu et al. 2011a). However, no significant correlation were found due to age in studies of As-exposed residents from Ghana, China and Cambodia (Asante et al. 2007; Mao et al. 2010; Phan et al. 2010).

The accumulation of Sb, Cd, Zn and Mn in hair and nails was not significantly influenced by age in current study. Another study found no effect of age on the Cd and Mn concentration (Chojnacka et al. 2006). For nails, it has been reported that young people (<45 years) had higher Sb concentration than elderly people (>45), and Zn was detected at lower levels in nail samples of young people (Chaudhary et al. 1995). Other studies have found significantly lower Zn hair concentrations in elderly subjects than in young control subjects (Vance et al. 1988; Rebacz et al. 2010). Age appeared to have significant influence on the concentrations of Th in hair in the current study, with decreasing Th levels with increasing age. Concentration of Th has been related to age in humans (Lucas et al. 1970), and a study found higher Th concentration in children than in elderly subjects (Rodushkin and Axelsson 2000). Soil is a probable source for Th as children generally have more outdoor activity and 'hand-to-mouth' behaviour (Ponzetta et al. 1998).

Arsenic was the only trace element in hair and nails from men for which there appeared to be an effect of smoking. Smokers had higher As concentration than non-smokers. Previous studies found no effect from smoking on the levels of Cd in hair (Chojnacka et al. 2006) or Zn levels in hair (Chiba and Masironi 1992). Another study found no significant differences in trace element concentrations between smokers and non-smokers (Rodushkin and Axelsson 2000). Exposure to As from smoking has been suggested to interact with high As levels in drinking water as an elevated risk of bladder cancer were observed among smokers that were exposed to As in drinking water compared with smokers with lower As drinking water exposure (Kapaj et al. 2006).

Tobacco smoking has been reported to influence the trace element concentrations of As, Cd, Cu, Pb, Mn and Zn in human tissues (Chiba and Masironi 1992; Mehra and Juneja 2005; Massadeh et al. 2011). Tobacco smoke contains a number of metals, including As, Cd, Pb and

Sb (Nordberg et al. 2007a), and the main sources of metal in cigarettes are tobacco as well as wrapping paper and filter (Mehra and Juneja 2005).

Adverse effects from metal absorption are modulated by the susceptibility of populations additionally to age, gender and smoking. Susceptible population concerning trace element absorption is caused by factors such as genetic polymorphism, synergetic effects between metals, bioavailability, allergies and diseases (Fairbrother et al. 2007). An example is carcinogenesis from As exposure which has been found to be correlated with malnutrition and protein diet deficiency (Roy and Saha 2002).

The element accumulation of As, Pb, Cu and Th in hair was influenced by gender and/or age, but the factors did not influence the element accumulation in nails. The concentration of As in men was significantly influenced by age and smoking in hair and nails respectively. Differences were more strongly affected by the village of habitation than any other factor for most elements, suggesting that other sources for exposure were minor compared to exposure via diet and/or drinking water.

5.6. Relationship between hair and nails

Correlation between hair and nail concentrations was found for the elements As, Cd, Zn and Th. There is limited knowledge of the relationships between trace elements in hair and nails in published literature, which has mainly focused on correlations between drinking water levels and/or other factors.

A study on hair-nail relationship found a significant positive correlation for As and for Sb (Vance et al. 1988). Controversially, it was found that individuals exhibiting high hair As levels did not necessarily have elevated As levels in nails (Ndiokwere 1985). The study surprisingly found that the exposed population with relatively high As levels in hair had lower As levels in nails. Another exposed population had significant correlation in hair and nail Pb levels, whereas no correlation was observed in the unexposed population (Mehra and Juneja 2003). The study also found no relationship of Cd concentration between hair and nails of exposed and unexposed subjects. The study of Vance et al. (1988) reported that concentrations of non-essential trace elements were positively correlated in hair and nail, whereas concentrations of essential elements were not correlated.

The existing knowledge about hair-nail relationships for trace elements is confusing. Half of the correlation analyses in the current study found no significant relationship between matched hair and nail samples. The results indicate that incorporation of trace elements is different for hair and nails, despite some similarities in chemical composition.

5.7 Evaluation of matrices

Hair and nails appeared to be the best indicator tissues for trace element exposure in the current study. The concentrations were above detection limits for all tissues, but the levels were generally higher in nail samples. Concentrations in blood were low, and the tissue was less useful as a indicator tissue in the current study as the most important aspect was to investigate chronic exposure rather than acute exposure.

No single matrix appears to be the most toxicologically relevant or the most sensitive biological indicator for all trace elements (He 2011). Hair and nails have the advantage of reflecting trace element levels during the time of formation, whereas blood provides the concentration at the time the sample was obtained (Gellein et al. 2008). Another benefit is the ease of collection, and the matrices do not require the same precautions for handling or storage as blood do. The limitations of hair and nails include the difficulty of collecting adequate sample from subjects with short hair and/or nails, and there are no well-defined reference ranges or health-based threshold levels for trace elements concentration (Orloff et al. 2009).

An approach to risk assessment is generally by comparison with ‘normal’ concentration ranges from unexposed populations. Normal ranges for the elements discussed above vary within published literature and the results obtained depended on the method used (Rodushkin and Axelsson 2000). External contaminants in water or dust can bind to proteins in hair and nails, resulting in an overestimation of trace element exposure (Orloff et al. 2009).

In spite of possible external contamination, the World Health Organization (WHO) and Environmental Protection Agency (EPA) has recommended hair as an important biological indicator matrix for environmental monitoring (Puchyr et al. 1998). The US EPA has concluded that hair is a representative tissue for biological monitoring of exposure to As, Sb, Cd, Pb and Cu (Hu and Brain 2004).

The use of nails in biomonitoring is not as widespread as hair, but the use has increased the recent decades (Oluwole et al. 1994). Nail concentration is generally viewed as an useful marker for exposure for elements such as As and Cd (He 2011). Concentrations of environmental contaminants have been found to be higher in nails than in hair, but the trend differs depending on the trace elements under study (Samanta et al. 2004). Toenails have been suggested to be less prone to contamination than fingernails (Orloff et al. 2009). It was presumably better to use fingernails in the current study as the villagers from Tarime District mainly walk barefoot or use open shoes, as do most Africans. As a biomarker for exposure, nails share many of the same advantages and limitations of hair biomonitoring. Nails reflect long-term exposure and incorporate trace elements proportional to intake or exposure. On the other hand, nail accumulation is not well characterized for some elements such as Cu, and nails may be contaminated through other sources than water and food, e.g. use of medications and nail polish (He 2011).

The advantage of blood as an indicator matrix is that it clearly eliminates external contamination, and it is a direct marker of internal dose (Gellein et al. 2008). The limitations include rapid clearance of trace elements and low concentrations compared to levels in hair and nails. Such factors have limited the use of blood biomonitoring for assessment of environmental exposure.

Hair, nails and blood have different advantages and limitations as biomonitor tissue for trace element exposure. Hair and nails reflect long term exposure of trace elements, and the concentrations are normally higher than in blood due to the high content of keratin (Orloff et al. 2009). Despite the limitations of each tissue, all have the potential as indicators for human trace element exposure.

5.8. Conclusions

The findings of current study indicate that villagers from Tarime District were exposed to higher levels of As than inhabitants of Dar es Salaam. The difference were most clearly observed for nails, but apparent for both tissues. Values were above unexposed subjects from other studies, and some individuals had higher concentrations than suggested upper normal level of As in hair and nail samples. There is a risk of health impairment in subjects based on comparison with As concentration in hair and nails from other exposed populations. Various skin lesions were

observed in some subjects, and there is a comprehensible need to clarify possible health effects associated with the high As concentrations observed.

There were not elevated concentrations of Sb, Cu or Cd in subjects from Tarime District compared to the reference group and other unexposed populations. Low hair and nail levels of Zn in subjects from Tarime district and Dar es Salaam indicate that some individuals may suffer health impairment associated with Zn deficiency, e.g. oral and prostate cancer. Zn deficiency may affect health impairments associated with As since low levels of Zn in hair and nails have been suggested to aggravate the toxicity of As.

Some subjects from Tarime District had higher hair and nails Mn concentrations than the reference group, and the levels in villagers were above the normal range for Mn. The observed concentrations of Mn in hair and/or nails have been associated with health impairments in other studies. Nail concentrations of Th were significantly higher in subjects from Tarime District than in the reference group, but no differences were found in hair samples. Both hair and nail samples from Tarime District had higher concentrations than found in previous studies of Th in human tissue.

Blood concentrations of some trace elements were different between subjects from Tarime District and Dar es Salaam, but blood values were generally lower in the current study than for other exposed and unexposed populations. The levels of As in blood were generally low for subjects in the current study than reported levels from other As exposed populations. The higher As levels in subjects from Dar es Salaam were expected to mainly consist of organic As species, which would not be of great concern as only intake of inorganic As has been related to adverse health effects.

Accumulation of As, Pb, Cu and Th in hair appeared to be influenced by gender and/or age, but the factors did not influence the element accumulation in nails. The concentration of As in men was significantly influenced by age and smoking in hair and nails respectively.

The results of the current study as well as dissimilar observations of hair-nail relationships from previous studies indicate that incorporation of trace elements is different for hair and nails. Hair and nails reflect long term exposure of trace elements, whereas blood reflects short-term exposure. Hair, nails and blood have different advantages and limitations as biomonitor tissues, but they all have the potential as indicators of human trace element exposure.

5.9. Future directions

The bioavailability of trace elements in ingested food and water are variable. Future research will be required to obtain a better knowledge of how specific factors and traditional cooking processes influence the trace element bioavailability. The impact of the factors age, gender and smoking habits should be considered to identify interactions among toxic trace elements. It is important to investigate several trace elements in human exposure studies as a deficiency of some elements, e.g. Zn and Mn, could modulate and possibly increase the toxicity of other elements, e.g. As.

More continuous monitoring studies are needed to develop a better understanding of the relationship between exposure to trace elements and adverse health effects. Government agencies and non-governmental organizations (NGO's) have done little research and monitoring of rural water. Total consumption of possible contaminated drinking water and dietary habits should be regularly considered mainly in population groups that are at risk of high trace element intakes.

The majority of epidemiological studies applying hair, nails and blood as biomonitor tissues for trace element exposure are aimed at evaluating the health effects associated with trace element exposure. Guidelines have been developed for concentration of many trace elements in drinking water, and this should also be done for levels in human tissues based on quantitative studies.

There is a need to develop social, technical and environmental sustainability and management of mining operations. Monitoring and source assessment are crucial whenever contamination from past or present mining activity is suspected. An evaluation of possible health effects of potentially exposed subjects from Tarime District is required, as well as accurately identification the sources for exposure to the trace elements.

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Appendices

Appendix 1 - Raw data of hair, nails and blood from all locations.

Appendix 2 - Map of the sample sites in North Mara in Table 1, data obtained from Almås et al. (2009).

Appendix 3 - Respond from Regional Committees for Medical Research Ethics (REK) on application to conduct the study.

Appendix 4 - Questionnaire (in Swahili) for the participants of the study.

Appendix 5 – Subject (anonymous) from Tarime District with skin lesions.

Appendix 1. Raw data of hair, nails and blood from all locations

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
1	1	1	0	44	0	1	0.0319	0.679	0.604	0.688	0.140	10.161	122.2	81.54	135.04	0.255
2	2	2	0	69	0	1	0.023	0.083	0.072	0.026	0.053	1.643	39.0			
3	3	1	0	83	0	1	0.0108	0.808	0.121	0.228	0.159	2.701	93.4	95.60	66.26	0.217
4	4	2	0	20	0	1	0.0075	0.201	0.072	0.006	0.041	0.319	94.6			
5	5	1	1	7	0	1	0.0199	0.340	0.130	0.044	0.125	1.657	124.5	11.07	10.76	0.085
6	6		1	65	0	1	0.0064	0.163	0.317	0.078	0.099	2.147	108.2	31.94	16.98	0.042
7	7	1	0	70	1	1	0.0132	0.277	0.064	0.080	0.069	2.276	54.2	7.28	18.28	0.023
8	8	1	1	38	0	1	0.0094	0.080	0.028	0.013	0.089	0.510	140.1	13.03	11.26	0.005
9	9	1	0	42	0	1	0.0091	0.275	0.029	0.054	1.248	8.218	93.9	40.76	12.12	0.025
10	10	1	0	32	1	1	0.0029	1.896	0.141	0.112	0.109	8.995	127.0	55.94	79.57	0.311
11	11	1	0	38	0	1	0.0056	0.379	0.066	0.084	0.414	4.222	118.7	19.66	15.39	0.025
12	12	3	0	46	0	1	0.0064	0.195	0.064	0.039	0.144	3.033	66.0	27.36	11.69	0.065
13	13	3	0	73	0	1	0.0078	0.175	0.035	0.085	0.075	1.357	151.2	31.90	20.34	0.034
14	14	3				1	0.0112	0.550	0.031	0.021	0.149	0.625	160.4	100.56	12.97	0.161
15	15	3				1	0.0103	0.120	0.028	0.035	0.067	0.599	98.3	28.90	11.93	0.099
16	16	3				1	0.0012	0.958	0.393	0.060	0.334	4.438	219.4	96.52	30.21	0.180
17	17	3	0	36	0	1	0.0075	0.112	0.107	0.014	0.045	0.890	195.0	37.78	8.92	0.036
18	18	3	1	30	0	1	0.0042	0.189	0.037	0.027	0.109	0.539	163.6	15.92	9.73	0.047
19	19	4	1	52	0	1	0.0106	0.276	0.125	0.112	0.141	1.102	124.1	129.84	8.89	0.109
20	20	5	0	47	1	1	0.0433	0.272	0.031	0.033	0.138	1.201	117.1	29.39	11.61	0.096
21	21	4	0	63	1	1	0.0198	0.222	0.084	0.042	0.084	1.667	69.9	25.13	8.81	0.045

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
22	22	4	1	74	0	1	0.005	0.054	0.015	0.005	0.052	0.553	200.7	7.42	12.26	0.021
23	23	5	0	35	0	1	0.0085	0.844	0.249	0.423	0.150	25.142	152.3	29.08	39.09	0.025
24	24	6	0	55	0	1	0.0012	0.295	0.086	0.041	0.185	1.717	81.0	28.22	17.05	0.076
25	25	6	0	40	0	1	0.0137	0.148	0.286	0.017	0.064	0.803	106.4	15.95	17.27	0.042
26	26	7	0	74	0	1	0.0048	0.170	0.128	0.015	0.095	1.517	119.8	6.01	9.34	0.049
27	27	6	0	32	0	1	0.0003	0.732	0.257	1.873	0.494	15.616	271.0	104.98	50.53	0.478
28	28	4	0	51	0	1	0.0019	0.066	0.266	0.000	0.082	0.852	174.7	10.78	19.79	0.028
29	29	6	0	45	0	1	0.0003	0.918	5.148	2.210	0.990	5.263	450.3	36.08	44.80	0.177
30	30	4	1	15	0	1	0.0026	0.116	0.067	0.030	0.144	0.908	155.1	18.79	12.46	0.072
31	31	5	0	63	0	1	0.0267	0.250	0.087	0.016	0.080	0.719	162.9	7.68	9.34	0.041
32	32	4	1	12	0	1	0.0021	0.042	0.190	0.167	0.092	1.162	135.4	31.63	16.44	0.038
33	33	6	0	80	0	1	0.0139	0.305	0.047	0.032	0.155	0.997	144.5	11.10	11.33	0.020
34	34	4	0	13	0	1	0.0036	0.226	0.041	0.094	0.233	2.490	61.7	24.53	17.70	0.067
35	35	1	1	42	0	1	0.011	0.443	0.220	0.083	0.139	7.253	74.6	23.89	20.85	0.069
36	36	1	1	60	0	1	0.008	0.164	0.037	0.047	0.147	2.701	133.0	16.64	18.18	0.015
37	37	4	1	70	0	1	0.0114	0.172	0.138	0.038	0.108	0.280	142.6	16.15	17.03	0.071
38	38	4	0	56	0	1	0.0496	0.374	0.261	0.090	0.077	2.842	99.2	26.31	17.28	0.076
39	39	5	0	36	0	1	0.0096	0.796	0.093	0.050	0.193	2.945	45.1	22.89	14.68	0.096
40	40	5	1	33	0	1	0.0057	0.850	0.236	0.222	0.140	5.103	43.2	24.10	11.19	0.036
41	41	5	0	13	0	1	0.008	0.420	0.045	0.054	0.112	3.139	128.4	13.92	10.29	0.034
42	42	6	1	45	0	1	0.0125	0.906	0.063	0.044	0.141	2.036	45.7	16.95	9.31	0.077
43	43	5	1	44	0	1	0.0281	0.779	0.136	0.065	0.113	4.692	66.7	21.53	10.16	0.080
44	44	4	0	45	1	1	0.0224	0.194	0.030	0.166	0.111	2.006	113.0	13.32	12.79	0.045
45	45	2	0	34	1	1	0.0145	2.351	0.075	0.020	0.120	2.130	93.8	10.03	12.82	0.037

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
46	46	2	0	52	1	1	0.0233	1.132	0.246	0.354	0.079	9.550	109.6	15.86	84.24	0.059
47	47	5	0	29	1	1	0.0101	0.548	0.111	0.045	0.142	2.920	130.5	40.57	21.25	0.101
48	48	7	1	46	0	1	0.007	0.281	0.178	0.020	0.235	1.011	108.7	25.68	10.27	0.100
49	49	7	1	11	0	1	0.0133	0.176	0.043	0.025	0.093	0.710	132.0	11.46	7.76	0.044
50	50	7	1	22	0	1	0.0088	0.331	0.128	0.047	0.198	3.391	82.0	18.97	12.62	0.079
51	51	7	0	5	0	1	0.0026	0.931	0.373	0.131	0.405	4.189	128.2	44.81	72.83	0.172
52	52	5	0	72	1	1	0.0188	0.790	0.115	0.046	0.069	3.903	102.6	7.62	25.40	0.079
53	53	5	0	11	0	1	0.004	2.318	0.452	0.050	0.255	4.165	154.5	38.07	57.99	0.425
54	54	5	0	8	0	1	0.0078	1.136	0.212	0.047	0.170	2.146	132.5	35.25	11.46	0.249
55	55	3	0	45	0	1	0.0127	0.112	0.175	0.037	0.138	0.579	140.2	158.59	9.97	0.056
56	56	3	0	45	0	1	0.0425	0.095	0.047	0.011	0.060	0.326	91.6	5.45	4.01	0.100
57	57	3	0	41	0	1	0.0009	4.235	1.638	0.260	1.013	5.428	2089.0	96.35	74.73	2.276
58	58	3	0	50	0	1	0.0082	1.857	0.364	0.146	0.249	2.600	579.1	34.46	18.80	0.344
59	59	2	0	58	0	1	0.0338	0.686	0.430	0.019	0.045	0.198	84.5			
60	60	3	0	8	0	1	0.0042	2.420	0.423	0.074	0.260	5.643	357.0	41.89	17.23	1.573
61	61	4	1	74	0	1	0.0026	0.321	0.578	0.050	0.189	0.923	205.6	9.82	9.73	0.102
62	62	4	1	28	0	1	0.0306	0.102	0.060	0.077	0.031	0.426	82.6	1.44	2.79	0.025
63	63	4	1	1	0	1	0.0036	0.958	0.445	0.115	0.631	2.261	404.2	40.32	15.34	1.288
64	1	1	0	44	0	2	0.028	0.290	0.045	0.011	0.084	0.609	85.5	7.49	2.98	0.297
65	2	2	0	69	0	2	0.0065	7.119	0.114	0.028	0.140	0.761	325.4			
66	3	1	0	83	0	2	0.0189	0.195	0.078	0.064	0.080	0.303	127.4	1.92	4.18	0.036
67	4	2	0	20	0	2	0.0333	0.412	0.119	0.014	0.059	0.288	46.9			
68	5	1	1	7	0	2	0.0302	0.174	0.063	0.099	0.057	0.591	118.7	3.24	6.30	0.055
69	6		1	65	0	2	0.0198	0.019	0.066	0.008	0.024	0.101	24.3	2.37	1.75	0.005

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
70	7	1	0	70	1	2	0.0231	0.224	0.109	0.050	0.165	0.987	134.3	37.69	15.89	0.088
71	8	1	1	38	0	2	0.007	0.222	0.245	0.086	0.093	3.595	90.8	25.09	7.37	0.064
72	9	1	0	42	0	2	0.0246	0.121	0.061	0.008	0.031	0.173	28.9	13.64	1.61	0.040
73	10	1	0	32	1	2	0.0085	4.439	1.845	0.694	0.761	28.009	456.8	169.57	138.85	0.343
74	11	1	0	38	0	2	0.048	0.111	0.056	0.011	0.022	1.210	22.6	5.74	2.99	0.021
75	12	3	0	46	0	2	0.0209	0.041	0.990	0.010	0.012	0.244	8.9	2.43	1.72	0.004
76	13	3	0	73	0	2	0.0396	0.373	0.053	0.174	0.119	1.566	126.7	85.31	11.48	0.176
77	14	3				2	0.02	0.479	0.071	0.025	0.126	0.386	90.0	24.41	4.42	0.408
78	15	3				2	0.0212	0.119	0.016	0.013	0.078	0.313	128.6	9.03	3.28	0.248
79	16	3				2	0.047	0.241	0.011	0.003	0.031	0.170	45.4	20.26	1.74	0.153
80	17	3	0	36	0	2	0.0322	0.780	0.027	0.012	0.059	0.402	75.4	12.22	2.85	0.286
81	18	3	1	30	0	2	0.0145	0.537	0.022	0.014	0.062	0.238	105.0	15.48	3.12	0.138
82	19	4	1	52	0	2	0.0094	0.267	0.030	0.008	0.050	0.286	43.8	3.70	2.61	0.108
83	20	5	0	47	1	2	0.051	0.670	0.022	0.012	0.129	0.704	87.5	37.29	2.96	0.618
84	21	4	0	63	1	2	0.0131	0.134	0.018	0.014	0.074	0.495	66.8	5.35	2.80	0.085
85	22	4	1	74	0	2	0.0047	0.231	0.078	0.024	0.059	2.031	155.7	16.56	13.00	0.149
86	23	5	0	35	0	2	0.053	0.470	0.052	0.008	0.066	0.512	85.3	14.12	3.66	0.138
87	24	6	0	55	0	2	0.0067	0.243	0.035	0.011	0.077	0.256	111.9	3.11	4.67	0.054
88	25	6	0	40	0	2	0.0148	0.425	0.053	0.023	0.115	0.786	94.8	13.21	3.48	0.187
89	26	7	0	74	0	2	0.0109	0.207	0.017	0.029	0.124	0.477	98.5	7.48	3.84	0.136
90	27	6	0	32	0	2	0.0056	0.194	0.137	0.125	0.108	0.959	99.9	15.13	5.02	0.192
91	28	4	0	51	0	2	0.0218	0.094	0.015	0.008	0.038	0.323	94.5	1.59	3.15	0.012
92	29	6	0	45	0	2	0.0078	0.376	0.053	0.018	0.125	0.547	76.6	9.60	3.34	0.260
93	30	4	1	15	0	2	0.003	0.164	0.027	0.009	0.065	0.273	95.9	15.46	3.50	0.118

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
94	31	5	0	63	0	2	0.0316	0.120	0.039	0.006	0.032	0.137	123.4	1.90	3.06	0.029
95	32	4	1	12	0	2	0.0036	0.216	0.030	0.025	0.156	0.520	86.1	18.88	3.33	0.538
96	33	6	0	80	0	2	0.045	0.286	0.017	0.009	0.071	0.251	108.4	12.07	2.62	0.222
97	34	4	0	13	0	2	0.0348	0.214	0.030	0.014	0.127	0.364	100.4	6.05	3.07	0.145
98	35	1	1	42	0	2	0.0364	0.658	0.047	0.021	0.131	0.637	45.9	10.00	2.33	0.226
99	36	1	1	60	0	2	0.0275	0.667	0.072	0.014	0.099	0.796	85.9	10.16	5.19	0.274
100	37	4	1	70	0	2	0.052	0.563	0.027	0.010	0.140	0.518	56.5	14.80	3.70	0.478
101	38	4	0	56	0	2	0.048	0.166	0.017	0.008	0.037	0.272	108.3	8.79	3.66	0.135
102	39	5	0	36	0	2	0.0176	0.490	0.026	0.012	0.342	0.461	69.5	12.56	3.40	0.415
103	40	5	1	33	0	2	0.0366	1.289	0.070	0.006	0.110	0.463	78.7	16.50	2.76	0.092
104	41	5	0	13	0	2	0.0232	0.254	0.019	0.041	0.076	0.199	126.2	5.09	3.55	0.083
105	42	6	1	45	0	2	0.0204	0.155	0.019	0.008	0.085	0.272	82.2	3.84	3.33	0.082
106	43	5	1	44	0	2	0.0383	0.701	0.031	0.024	0.091	0.778	105.4	23.08	2.98	0.193
107	44	4	0	45	1	2	0.048	0.275	0.033	0.138	0.064	0.566	96.0	10.40	3.74	0.095
108	45	2	0	34	1	2	0.0495	14.317	0.189	0.020	0.234	1.700	486.6			
109	46	2	0	52	1	2	0.0493	2.017	0.144	0.032	0.089	0.918	556.3			
110	47	5	0	29	1	2	0.0283	1.863	0.019	0.009	0.061	0.455	73.0	4.83	3.80	0.114
111	48	7	1	46	0	2	0.0407	0.326	0.023	0.008	0.134	0.526	78.8	14.71	3.95	0.256
112	49	7	1	11	0	2	0.0405	0.135	0.017	0.005	0.106	0.164	105.3	13.60	4.14	0.058
113	50	7	1	22	0	2	0.0244	0.485	0.069	0.014	0.126	0.500	68.0	13.50	3.57	0.169
114	51	7	0	5	0	2	0.0135	0.447	0.043	0.010	0.162	0.679	72.0	11.12	3.83	0.198
115	52	5	0	72	1	2	0.0503	0.865	0.031	0.008	0.057	0.326	96.0	6.33	3.12	0.162
116	53	5	0	11	0	2	0.0203	1.427	0.031	0.008	0.102	0.415	77.9	7.44	2.28	0.269
117	54	5	0	8	0	2	0.0105	3.200	0.355	0.019	0.188	0.850	91.6	19.13	5.48	0.607

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
118	55	3	0	45	0	2	0.034	0.209	0.055	0.031	0.080	0.723	96.2	9.08	4.07	0.120
119	56	3	0	45	0	2	0.048	0.355	0.015	0.021	0.040	0.155	133.3	11.80	3.78	0.021
120	57	3	0	41	0	2	0.0087	0.295	0.062	1.009	1.093	5.881	103.4	5.74	3.30	0.051
121	58	3	0	50	0	2	0.0172	1.383	0.223	0.016	0.282	4.882	112.7	19.47	4.90	0.112
122	59	2	0	58	0	2	0.0579	4.466	0.162	0.077	0.682	4.510	343.5			
123	60	3	0	8	0	2	0.0182	1.323	0.042	0.024	0.437	2.220	92.1	317.25	11.11	0.860
124	61	4	1	74	0	2	0.0055	0.275	0.099	0.032	0.113	0.820	143.0	15.65	5.28	0.289
125	62	4	1	28	0	2	0.0176	0.268	0.025	0.011	0.057	1.944	99.4	7.40	3.18	0.138
126	63	4	1	1	0	2	0.0029	0.405	0.114	0.024	0.117	0.550	125.4	12.30	9.71	0.223
127	1	1	0	44	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0037	0.5817	0.0005	0.0641	0.0000
128	2	2	0	69	0	3	0.5	0.0233	0.2430	0.0026	4.0132	0.4658	139.71			
129	3	1	0	83	0	3	0.5	0.0003	0.0007	0.0000	0.0001	0.0020	0.4611	0.0006	0.1062	0.0000
130	4	2	0	20	0	3	0.5	0.0200	0.1431	0.0044	0.0328	0.5295	107.10			
131	5	1	1	7	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0016	0.3630	0.0013	0.0863	0.0000
132	6		1	65	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0020	0.3510	0.0005	0.0685	0.0000
133	7	1	0	70	1	3	0.5	0.0002	0.0007	0.0001	0.0001	0.0047	0.5721	0.0005	0.0639	0.0000
134	8	1	1	38	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0028	0.4093	0.0013	0.0742	0.0000
135	9	1	0	42	0	3	0.5	0.0002	0.0011	0.0000	0.0001	0.0045	0.4680	0.0007	0.0658	0.0000
136	10	1	0	32	1	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0034	0.4089	0.0021	0.0871	0.0000
137	11	1	0	38	0	3	0.5	0.0002	0.0005	0.0000	0.0001	0.0055	0.4195	0.0008	0.0559	0.0000
138	12	3	0	46	0	3	0.5	0.0002	0.0009	0.0000	0.0001	0.0018	0.5131	0.0007	0.0806	0.0000
139	13	3	0	73	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0012	0.3390	0.0009	0.0838	0.0000
140	14	3				3	0.5	0.0003	0.0010	0.0000	0.0002	0.0011	0.4656	0.0013	0.0713	0.0000
141	15	3				3	0.5	0.0002	0.0006	0.0000	0.0001	0.0006	0.2850	0.0006	0.0722	0.0000

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
142	16	3				3	0.5	0.0003	0.0006	0.0000	0.0001	0.0013	0.4919	0.0008	0.0728	0.0000
143	17	3	0	36	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0016	0.3915	0.0005	0.0731	0.0000
144	18	3	1	30	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0012	0.3122	0.0009	0.0960	0.0000
145	19	4	1	52	0	3	0.5	0.0002	0.0007	0.0000	0.0002	0.0017	0.3722	0.0010	0.0698	0.0000
146	20	5	0	47	1	3	0.5	0.0002	0.0006	0.0001	0.0002	0.0021	0.3253	0.0012	0.0751	0.0000
147	21	4	0	63	1	3	0.5	0.0003	0.0006	0.0000	0.0002	0.0055	0.3949	0.0012	0.0734	0.0000
148	22	4	1	74	0	3	0.5	0.0002	0.0006	0.0000	0.0002	0.0015	0.4128	0.0006	0.0674	0.0000
149	23	5	0	35	0	3	0.5	0.0002	0.0006	0.0001	0.0001	0.0049	0.4585	0.0008	0.0618	0.0000
150	24	6	0	55	0	3	0.5	0.0002	0.0007	0.0000	0.0001	0.0014	0.4109	0.0012	0.0574	0.0000
151	25	6	0	40	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0014	0.4026	0.0011	0.0573	0.0000
152	26	7	0	74	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0011	0.3416	0.0006	0.0720	0.0000
153	27	6	0	32	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0046	0.5317	0.0005	0.0563	0.0000
154	28	4	0	51	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0011	0.4642	0.0005	0.0608	0.0000
155	29	6	0	45	0	3	0.5	0.0004	0.0005	0.0000	0.0001	0.0023	0.2002	0.0013	0.0772	0.0000
156	30	4	1	15	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0012	0.5216	0.0008	0.0823	0.0000
157	31	5	0	63	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0022	0.4636	0.0006	0.0724	0.0000
158	32	4	1	12	0	3	0.5	0.0003	0.0007	0.0000	0.0001	0.0005	0.2666	0.0008	0.0811	0.0000
159	33	6	0	80	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0012	0.3532	0.0010	0.0639	0.0000
160	34	4	0	13	0	3	0.5	0.0002	0.0007	0.0000	0.0001	0.0024	0.3145	0.0014	0.0796	0.0000
161	35	1	1	42	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0015	0.2911	0.0005	0.0618	0.0000
162	36	1	1	60	0	3	0.5	0.0002	0.0005	0.0000	0.0001	0.0013	0.4252	0.0009	0.0836	0.0000
163	37	4	1	70	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0011	0.3213	0.0011	0.0783	0.0000
164	38	4	0	56	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0021	0.4327	0.0006	0.0691	0.0000
165	39	5	0	36	0	3	0.5	0.0003	0.0005	0.0000	0.0002	0.0014	0.5778	0.0009	0.0892	0.0000

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
166	40	5	1	33	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0026	0.3363	0.0009	0.0726	0.0000
167	41	5	0	13	0	3	0.5	0.0003	0.0006	0.0000	0.0002	0.0033	0.3454	0.0010	0.0668	0.0000
168	42	6	1	45	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0027	0.4315	0.0008	0.0926	0.0000
169	43	5	1	44	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0016	0.4136	0.0020	0.0784	0.0000
170	44	4	0	45	1	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0022	0.3318	0.0008	0.0584	0.0000
171	45	2	0	34	1	3	0.5	0.0278	0.1452	0.0220	0.8228	0.5535	89.888			
172	46	2	0	52	1	3	0.5	0.0002	0.0006	0.0001	0.0001	0.0063	0.4292	0.0006	0.0640	0.0000
173	47	5	0	29	1	3	0.5	0.0003	0.0010	0.0001	0.0001	0.0042	0.4414	0.0025	0.0740	0.0000
174	48	7	1	46	0	3	0.5	0.0003	0.0007	0.0000	0.0001	0.0011	0.2775	0.0007	0.0727	0.0000
175	49	7	1	11	0	3	0.5	0.0002	0.0006	0.0000	0.0005	0.0022	0.3182	0.0010	0.0851	0.0000
176	50	7	1	22	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0012	0.3527	0.0006	0.0613	0.0000
177	51	7	0	5	0	3	0.5	0.0002	0.0007	0.0000	0.0001	0.0014	0.2835	0.0011	0.0866	0.0000
178	52	5	0	72	1	3	0.5	0.0002	0.0005	0.0001	0.0001	0.0025	0.4320	0.0006	0.0745	0.0000
179	53	5	0	11	0	3	0.5	0.0002	0.0006	0.0000	0.0002	0.0025	0.2970	0.0010	0.0707	0.0000
180	54	5	0	8	0	3	0.5	0.0003	0.0007	0.0000	0.0001	0.0028	0.3860	0.0008	0.0764	0.0000
181	55	3	0	45	0	3	0.5	0.0002	0.0007	0.0000	0.0002	0.0007	0.3158	0.0011	0.0785	0.0000
182	56	3	0	45	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0013	0.4732	0.0005	0.0648	0.0000
183	57	3	0	41	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0009	0.4539	0.0006	0.0644	0.0000
184	58	3	0	50	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0041	0.4072	0.0007	0.0741	0.0000
185	59	2	0	58	0	3	0.5	0.0002	0.0006	0.0000	0.0001	0.0025	0.2953	0.0009	0.0708	0.0000
186	59	2	0	58	0	3	0.5	0.0002	0.0005	0.0000	0.0001	0.0017	0.5171	0.0010	0.0597	0.0000
187	61	4	1	74	0	3	0.5	0.0002	0.0013	0.0000	0.0002	0.0009	0.3959	0.0008	0.0848	0.0000
188	62	4	1	28	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0014	0.4423	0.0023	0.0823	0.0000
189	63	4	1	1	0	3	0.5	0.0002	0.0040	0.0000	0.0001	0.0007	0.2655	0.0015	0.1182	0.0000

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
190						3	0.5	0.0003	0.0010	0.0000	0.0001	0.0009	0.5685	0.0005	0.0588	0.0000
191	47	5	0	29	1	3	0.5	0.0003	0.0007	0.0001	0.0001	0.0033	0.4158	0.0022	0.0840	0.0000
192	38	4	0	56	0	3	0.5	0.0003	0.0006	0.0000	0.0002	0.0021	0.4229	0.0006	0.0738	0.0000
193	60	3	0	8	0	3	0.5	0.0003	0.0006	0.0000	0.0001	0.0007	0.3404	0.0014	0.0762	0.0000
194	2	2	0	69	0	3	0.5	0.0003	0.0007	0.0000	0.0001	0.0024	0.4709	0.0006	0.0842	0.0000
195			1			3	0.5	0.0003	0.0005	0.0000	0.0001	0.0026	0.2540	0.0009	0.0917	0.0000
196			1			3	0.5	0.0003	0.0012	0.0000	0.0001	0.0009	0.2389	0.0009	0.0920	0.0000
197	37	4	1	70	0	3	0.5	0.0004	0.0006	0.0000	0.0001	0.0010	0.2849	0.0011	0.0695	0.0000
198	64	8	1	29	0	3	0.5	0.0343	0.2236	0.0034	0.0280	0.2954	87.578			
199	65	8	1	49	0	3	0.5	0.0003	0.0011	0.0000	0.0001	0.0013	0.3465	0.0006	0.0666	0.0000
200	66	8	0	31	0	3	0.5	0.0004	0.0013	0.0000	0.0001	0.0015	0.3753	0.0006	0.0458	0.0000
201	67	8	1	24	0	3	0.5	0.0003	0.0011	0.0000	0.0001	0.0015	0.3329	0.0006	0.0660	0.0000
202	68	8	1	42	0	3	0.5	0.0004	0.0021	0.0000	0.0001	0.0018	0.3826	0.0006	0.0525	0.0000
203	69	8	0	39	0	3	0.5	0.0994	0.2600	0.0073	8.4992	0.3349	140.02			
204	70	8	0	26	0	3	0.5	0.0003	0.0011	0.0000	0.0001	0.0018	0.5054	0.0006	0.0587	0.0000
205	71	8	0	45	0	3	0.5	0.0006	0.0011	0.0000	0.0001	0.0036	0.3884	0.0007	0.0647	0.0000
206	72	8	0	27	0	3	0.5	0.0006	0.0009	0.0000	0.0001	0.0029	0.4804	0.0006	0.0529	0.0000
207	73	8	0	36	0	3	0.5	0.0015	0.0012	0.0000	0.0001	0.0014	0.5970	0.0006	0.0637	0.0000
208	64	8	1	29	0	1	0.002	0.0042	0.0906	0.0864	0.0188	0.9139	47.428			
209	65	8	1	49	0	1	0.000	0.0810	1.1734	0.3909	0.6811	28.142	260.68	8.0643	629.08	-0.0571
210	66	8	0	31	0	1	0.000	0.0720	0.1391	0.1040	0.2794	6.8154	95.870	1.0394	17.396	-0.0761
211	67	8	1	24	0	1	0.000	0.0540	0.8866	0.6268	0.4715	55.148	90.824	5.6450	267.18	-0.1142
212	68	8	1	42	0	1	0.004	0.0450	0.3332	0.4408	0.4366	36.664	95.568	9.5428	147.90	0.0730
213	69	8	0	39	0	1	0.001	0.0499	0.2247	0.0238	0.1370	2.8835	191.34	1.0670	16.223	-0.0264

Test no.	Pers-on no.	Loca tion	Sex (M0-F1)	Age	Smoker (N0,Y1)	Test (H1,N2, B3)	In-weight (g)	As (ug/g)	Sb (µg/g)	Cd (ug/g)	Mo (ug/g)	Pb (ug/g)	Zn (ug/g)	Mn UMB (ug/g)	Cu UMB (µg/g)	Th UMB (µg/g)
214	70	8	0	26	0	1	0.000	0.0020	0.0056	0.0040	0.0000	0.0415	0.9444			
215	71	8	0	45	0	1	0.001	0.4051	0.1695	0.0390	0.1179	2.8824	132.31	4.9192	16.571	-0.0286
216	72	8	0	27	0	1	0.001	0.2562	0.2724	0.0538	0.2894	1.7301	108.75	1.3540	13.873	0.0958
217	73	8	0	36	0	1	0.001	0.5002	0.7021	0.1205	0.1929	12.220	167.56	49.911	20.331	0.0000
218	64	8	1	29	0	2	0.007	0.0617	0.0851	0.0399	0.1576	4.4641	105.76	2.9558	9.7839	0.0079
219	65	8	1	49	0	2	0.012	0.0261	0.5385	0.0125	0.0142	1.1702	119.05	0.6446	7.7105	0.0009
220	66	8	0	31	0	2	0.019	0.0572	0.0338	0.0360	0.0217	0.7471	98.298	0.6573	4.1104	0.0030
221	67	8	1	24	0	2	0.012	0.0469	0.1225	0.0398	0.0325	1.3399	106.68			
222	68	8	1	42	0	2	0.006	0.1704	0.1014	0.0523	0.0917	3.3120	92.575	15.243	6.5828	0.0734
223	69	8	0	39	0	2	0.053	0.0756	0.0623	0.0101	0.0186	0.5324	109.91	1.3491	4.3790	0.0048
224	70	8	0	26	0	2	0.046	0.1117	0.0718	0.0209	0.0149	0.6209	128.81	0.7876	4.5109	0.0048
225	71	8	0	45	0	2	0.054	0.3817	0.0421	0.0129	0.0470	0.6850	109.92	6.9848	3.7591	0.0708
226	72	8	0	27	0	2	0.005	0.0251	0.0147	0.0040	0.0133	0.1156	36.720			
227	73	8	0	36	0	2	0.070	0.0680	0.0171	0.0020	0.0126	0.0923	117.21	4.9334	4.0749	0.0164

Appendix 2. Map of the sample sites in North Mara in Table 1, data obtained from Almås et al. (2009).

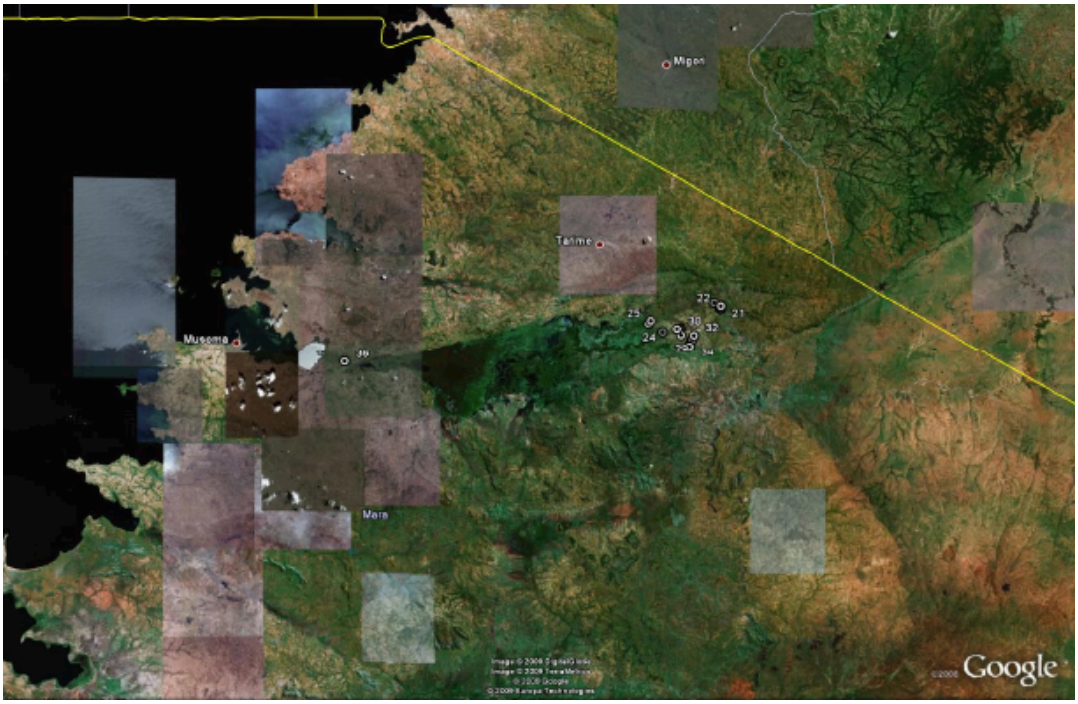

Image from Google Earth indicate the sampling sites near Tarime city and the control site 36.

Image from Google Earth indicting the sampling sites in east of Tarime. The installations are currently covering a much larger area than shown in the image. Site 36 is outside this image.

Appendix 3 - Respond from Regional Committees for Medical Research Ethics (REK) on application to conduct the study.

UNIVERSITETET I OSLO
DET MEDISINSKE FAKULTET

Professor Ketil Hylland
Biologisk institutt
Pb 1066, Blindern
Internpost

Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst C (REK Sør-Øst C)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: 22 84 46 67

E-post: post@helseforskning.etikkom.no
Nettadresse: http://helseforskning.etikkom.no

Dato: 29.06.2010
Deres ref.:
Vår ref.: 2010/1605 (oppgis ved henvendelse)

Analyse av metaller i vev fra mennesker, Nord-Tanzania

Vi viser til søknad mottatt til frist 27.05.2010om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden er blitt vurdert av Regional komité for medisinsk og helsefaglig forskningsetikk i henhold til lov av 20. juni 2008 nr. 44, om medisinsk og helsefaglig forskning (helseforskningsloven) kapittel 3, med tilhørende forskrift om organisering av medisinsk og helsefaglig forskning av 1. juli 2009 nr 0955.

Barrick Gold's North Mara Gold Mine ligger ved Tarime distriktet, ved elven River Tigithe, Nordvest-Tanzania. Gruven utvinner gull, og virksomheten har vært i gang siden 1998. Det er data som tyder på at River Tigithe blir tilført sporelementer som en konsekvens av gruvevirksomheten. Denne studien innebærer undersøkelser av lokalbefolkningen i det eksponerte området, samt av en kontrollgruppe, til sammen ca 75 personer. Hår-, negl- og blodprøver samles inn, og analyseres for utvalgte metaller ved Universitetet for miljø- og biovitenskap.

Prosjektleder: Dr. scient. Ketil Hylland
Forskningsansvarlig: Biologisk institutt, UiO

Etter søknaden fremstår ikke prosjektet som et medisinsk og helsefaglig forskningsprosjekt, og faller derfor utenfor komiteens mandat, jf. helseforskningslovens § 2.

Vedtak:
Prosjektet er ikke fremleggelsespliktig, jf. helseforskningslovens § 10, jf. helseforskningslovens § 4 annet ledd.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages til Den nasjonale forskningsetiske komité for medisin og helsefag, jf. Forvaltningslovens § 28 flg. Eventuell klage sendes til REK Sør-Øst. Klagefristen er tre uker fra mottak av dette brevet.

Med vennlig hilsen

Arvid Heiberg (sign.)
professor dr. med.
leder

Tor Even Svanes
seniorrådgiver

Appendix 4 - Questionnaire (in Swahili) for the participants of the study

Form No._____

FORM YA MAKUBALIANO YA KUTOA SAMPLI KWA AJILI YA UTAFITI

(Ijazwe kwa kila mshiriki)

1. Mimi _____(*majina matatu*) *nimekubali kwa hiyari yangu bila kushawishiwa na mtu kushiriki katika utafiti huu.*

2. Umri_____(Miaka)

3. Jinsia_____(Me/Ke)

4. Kijiji alichotoka_____

5. Amekaa hapo kijijini kwa muda gani? _____ (mwaka

6. Kazi yake _____

7. Anavuta sigara ____(Ndiyo/Hapana)

Sahihi _____

Appendix 5 - Subject (anonymous) from Tarime District with skin lesion

Photo: John Magufu